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THE ELECTROCHEMICAL FORMULATION OF THE IRREVERSIBLE REDUCTION AND OXIDA- TION OF ORGANIC COMPOUNDS

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The electrochemical study of reversible oxidation-reduction systems composed of organic compounds has been a fruitful field of inquiry during the last five years. These investigations were ably reviewed by Wm. Mansfield Clark (4) in this JOURNAL a little more than a year ago. The present paper is in the nature of a companion article dealing with some of those many oxidation or reduction reactions which proceed readily in solution but which are not strictly reversible. Recent work has indicated that certain of these may be profitably considered from the electrochemical standpoint although, of course, the usual electrochemical equations do not apply. It is the purpose of this review to summarize these recent efforts and to discuss their extension to other examples of irreversible oxidation or reduction reactions, pointing out certain probable limitations. Since the object of this paper is to consider only how far the electrochemical concepts of "potential" may be useful in interpreting such reactions, I shall make no attempt to review all the recent work in the field of oxidation and reduction and shall omit a detailed consideration of the subject of electrolytic reactions. I shall also have occasion to consider a few only of the many important results connected with oxidation which have been obtained recently in the field of biochemistry. When one considers the bewildering mass of information which falls under the head of irreversible oxidation or reduction, it is obvious that in a single article one could only hope either to deal with a small subdivision of this field or to restrict oneself to viewing the whole from a particular angle. I have chosen the latter course; to what

extent this viewpoint is of value is a problem for the future. The beginnings are as yet so small that one can only suggest the general lines along which the next advances will be made.

GENERAL INTRODUCTION

At the outset of this discussion it is important to have in mind clearly the distinction between the two different types of reduction or oxidation processes which are commonly designated by the terms reversible and irreversible. We have certain rather peculiar compounds such as the quinones, the indophenols, and sulfonated indigos, which in solution with their reduction products (hydroquinone, leuco indigo sulfonate, etc.) form systems¹ which are strictly reversible under ordinary conditions. An inert electrode immersed in a solution containing the two components has a definite and reproducible potential which is a logarithmic function of the relative concentrations of the oxidized and reduced substances (4, 6I). Since these potentials are generally a function of the hydrogen ion concentration one must be careful to employ buffered solutions of known pH. If the components of two such reversible systems are mixed, a rapid reaction takes place which comes to a definite equilibrium; the extent of this reaction can be predicted from the oxidation-reduction potentials of the two systems. For example, a solution of quinone in dilute acid is rapidly reduced by titanous chloride or a dilute solution of hydroquinone is oxidized by potassium dichromate; the difference in potential in both instances is so great that the reactions go to practical completion although a definite and calculable amount of quinone and hydroquinone is present in the equilibrium mixture. On the other hand, the action of monochloroquinone on a solution of hydroquinone is incomplete, since the normal potentials of the quinone-hydroquinone and chloroquinone-chlorohydroquinone systems are very near each other. The potentials which determine the extent of such interactions of reversible systems are the normal poten-

¹A mixture of the oxidized and reduced forms of an organic compound (in solution) will be referred to as a *system* throughout this paper.

tials; one can not define or measure the potential of a single substance such as quinone.

A very different picture is presented by the behavior of such substances as unsaturated 1,4 diketones, certain azo dyes and nitro compounds. No significant potentials can be measured in a system composed of dibenzoyl ethylene ($C_6H_5COCH = CHCOC_6H_5$) and dibenzoyl ethane ($C_6H_5COCH_2CH_2COC_6H_5$) or in a mixture of an azo dye and its final reduction products which are the result of a cleavage of the molecule. Although the addition of titanous chloride to a solution of dibenzoyl ethylene rapidly reduces this substances to dibenzoyl ethane, it is impossible to reoxidize dibenzoyl ethane by the addition of even such strong oxidizing agents as potassium dichromate. Similarly, we know of no procedure by which it is possible to go from the cleavage products of an azo dye back to the dyestuff itself.

Still another example is afforded by aliphatic aldehydes which are readily oxidized in alkaline solution to acids, but the reaction can not be reversed by the action of even the most powerful reducing agents available in aqueous solution. Cases of oxidation or reduction in which the reaction proceeds readily in only one direction have been designated as irreversible, meaning thereby irreversible under the particular conditions employed in the experiment.

It is, of course, entirely conceivable that in certain solvents or in the presence of a suitable catalyst such a pair of substances as dibenzoyl ethylene and dibenzoyl ethane might behave like quinone and hydroquinone. As a practical matter no such catalysts have as yet been found² (the usual platinum catalysts seem to be without effect) and the behavior of a variety of substances seems to be the same in water, alcohol and acetone.

In the last analysis the distinction between reversible and irreversible processes is perhaps only one of rates. Quinone and the dyestuffs react very rapidly with soluble reducing agents and their reduction products also react very rapidly with soluble oxidizing agents. Equilibrium is therefore established in solu-

² Certain biochemical catalysts which may constitute an exception to this statement are discussed in a later section of this paper.

tion at room temperature in the course of a few seconds or few minutes when we are dealing with these compounds. On the other hand, while the reduction of dibenzoyl ethylene proceeds very rapidly, reoxidation of dibenzoyl ethane solutions is so slow that it has never been experimentally observed. Given infinite time dibenzoyl ethane and a soluble oxidizing agent might come to just as definite an equilibrium as hydroquinone and an oxidizing agent, but since in the laboratory we are concerned with relatively short intervals of time the distinction between hydroquinone and dibenzoyl ethane is significant. This distinction between reversible and irreversible changes is of the utmost importance, and will be emphasized throughout this review; certain experimental criteria for differentiating the two types of reactions will be suggested later. *The term oxidation-reduction potential has meaning only when applied to systems which are strictly reversible.* Unless the potential of an inert electrode is a logarithmic function of the concentration of both components of the system (the oxidized and reduced compounds) the presumption must be that the system is irreversible. The reduction or oxidation of one component of such irreversible systems can not be formulated in terms of a true oxidation-reduction potential.

If it be granted that there is no direct method of measuring oxidation-reduction potentials of irreversible systems, the question arises as to the possibility of studying such changes quantitatively. If we have a certain substance, for example an azo dye, which is irreversibly reduced by soluble reducing agents (with cleavage of the molecule) the general experience of organic chemists would lead us to believe that this process is in some way conditioned by the oxidation-reduction potential of the reducing agent. Qualitatively the idea of "powerful" reducing agents and "mild" reducing agents has long been in vogue. Is this concept sound? The problem is really this: is the rate of an irreversible process governed by the free energy change involved? In other words, will the speed of reduction of a substance be a function of the oxidation-reduction potential of the reagent employed? Before considering the experimental evidence which

has been collected in the last few years to answer this question, it will be well to outline some of the general principles involved.

It is now usually recognized (in spite of statements to the contrary in the older textbooks of physical chemistry) that there is, in general, no necessary relationship between the speed and the free energy change of a reaction. Thus, if two different reducing agents with the same potential react with the oxidized compound A which is *reversibly* reduced in solution, the speed at which equilibrium is attained may be very different, though the final composition of the equilibrium in regard to A and its reduction product will be identical in both cases. There are many facts which can be quoted to support the contention that in a great number of instances free energy changes and rates of reaction do not run parallel. I am indebted to Professor G. N. Lewis for the apt illustration of this point which a consideration of the process of racemization supplies. Here the free energy change from one pure antipode to another is zero; yet with many compounds racemization is very rapid, while with others it is very slow.

There is obviously one set of conditions under which the speed of a reaction must be governed by a free energy change,—that is, when some equilibrium process controls the amount of material undergoing a subsequent irreversible transformation. This can be expressed in general terms for the irreversible transfer of two hydrogen atoms as follows:



In the above scheme the total process with which we are concerned may be represented as an irreversible transformation of A into P. If this proceeds through the formation of AH_2 in a reaction which reaches equilibrium under the conditions of the experiment, the rate of the irreversible step will be a function of the amount of AH_2 in solution. This in turn will be different according as we use BH_2 (eq. 1) or another substance of different

potential, $B'H_2$ (eq. 2), as our reducing agent. (An algebraic formulation of this is given on page 17). It will be shown that similar mechanisms can be suggested and in some instances fairly well established for those cases in which processes of oxidation and reduction are dependent on the potential of the oxidizing or reducing agent employed. Where no such reversible step controls the rate, the potential of the reagent is probably of no significance.

Usually it is necessary to decide by a series of experiments whether or not a given process of reduction or oxidation is governed by the potential of the reagent employed. Such experiments will now be considered; where the potential of the reagent is the governing factor, they lead to quantitative methods of formulating irreversible reductions or oxidations which are more definite and satisfactory than those implied by the use of such terms as "powerful reducing agent" and "easily reduced substances."

IRREVERSIBLE REDUCTIONS

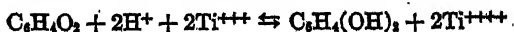
In order to study the relation between the speed of an irreversible reduction and the potential of the reducing agent, it is necessary to perform a number of experiments in which a graded series of reagents of known potential are employed. To determine the rate, one could obviously analyze the solution from time to time but this process is cumbersome and the reactions are often very rapid. A much more convenient method is that based on the observation of the color change of the reducing agent. For example, if leuco methylene blue is used, and the solution remains colorless throughout the experiment it is obvious that no reaction has taken place since the oxidation product of the leuco compound,—methylene blue itself,—is intensely colored. Dr. Clark (6) and his collaborators at the Hygienic Laboratory have prepared a whole series of dyestuffs and very carefully measured their potentials in solutions of varying acidity. Such compounds may be considered as forming a series of oxidation-reduction indicators. The use of such indicators promises to develop a whole new field in the quan-

titative study of the oxidation and reduction processes taking place in living organisms for which purpose they were particularly designed.

A third method which is less convenient than the use of indicators, but which has a somewhat greater range of applicability in the organic laboratory, involves the use of a series of reversible systems and an electrochemical cell very similar to that employed in the determination of the potential of reversible systems. In this method an equimolecular mixture of the reducing agent and its oxidized form is placed in the cell and the potential of an inert electrode measured in the usual way. The substance under examination which is to be irreversibly reduced is added. If it reacts with the reducing agent when introduced into the solution, changes in the composition of the reversible system will result and will affect the potential. The rate of change of potential will be a function of the speed of the irreversible reduction; if no change takes place one can conclude there has been no reaction. It is necessary to use as reducing agents only those substances which form strictly reversible systems which rapidly come to equilibrium. A number of organic compounds and certain inorganic compounds are suitable, but the electrode equilibria adjust too slowly in the case of a number of common reducing agents, such as stannous chloride. A series of suitable reversible systems (including many of Dr. Clark's dyes) are shown in figure 1; the potential of an equimolecular mixture of the two components in dilute solution (the "normal" potential) is plotted against the hydrogen ion concentration. It should be noted that the organic reagents for the most part are designated by the name of the oxidized form; as a *reducing* agent the reduced form would be employed.

The reader is referred to Dr. Clark's article for an exposition of the principles underlying the change of potential of reversible organic systems with change of hydrogen ion concentration. It is immaterial for our purpose whether the reduced and oxidized components of these reversible systems are actually hydrogenated and dehydrogenated organic compounds of the type BH_2 and B , or salts derived from one or the other of these, or

inorganic ions. It is necessary only that these systems are reversible in the sense that in solution they rapidly react with one another coming to a definite equilibrium whose composition is a function of their respective potentials. It is obvious that since we are working always with solutions containing water that the requisite hydrogen in many instances must come from the hydrogen ion as illustrated by the *reversible* interaction of quinone and titanous chloride.



Whether the fundamental reaction of reversible organic systems is a transference of hydrogen atoms or of electrons is an interesting problem but one with which we are not here concerned.

The electrochemical method just outlined has been used in studying the irreversible reduction of a number of azo dyes, nitro compounds and unsaturated 1,4 diketones and related substances in dilute acid and in buffered alkaline solutions. No exceptions have been found to the statement that the reduction in homogeneous solution of such compounds is a function of the potential of the reducing agent. This generalization can hardly be the result of accident, since the reagents include a number of different types of organic and inorganic substances. It may be concluded, therefore, that the action of soluble reducing agents on these compounds may be formulated in terms of the potential of the reagent. This is conveniently done by considering a hypothetical system which would just cause "appreciable reduction" (20 to 30 per cent, in thirty minutes); the potential of such a "critical reagent" may be considered as "the apparent reduction potential" of the substance being investigated under the conditions employed.

In most instances it has been found necessary to estimate "the apparent reduction potential" (A.R.P.) as lying somewhere between the potentials of two reagents about 100 millivolts apart, one of which causes less than 5 per cent reduction in thirty minutes, the other of which causes almost instantaneous reduction. In a few instances "border line cases," have been observed corresponding closely to the action of the hypothetical

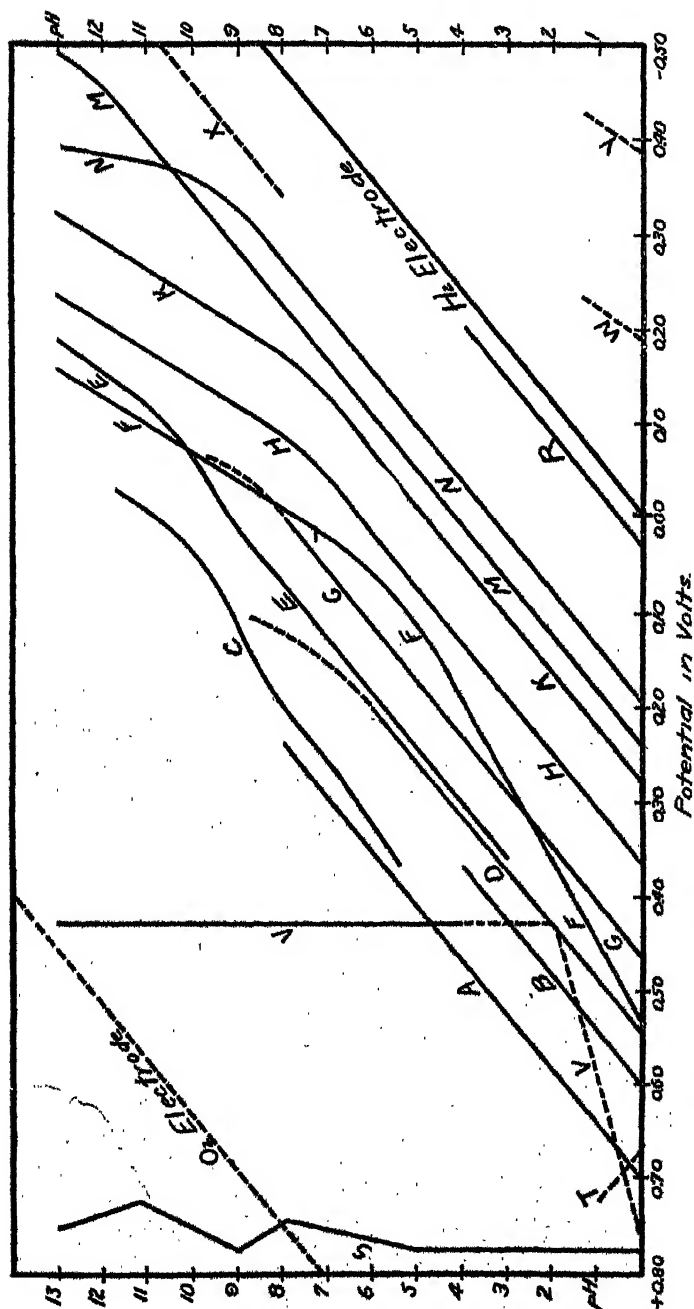


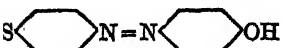
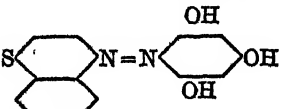
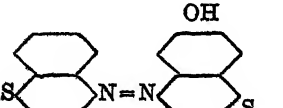
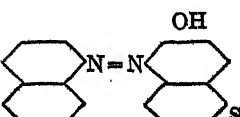
FIG. NO. 1. AL OXIDATION-REDUCTION POTENTIALS AT 25° OF SOME REAGENTS IN BUFFER SOLUTIONS OF VARYING pH (Concentrations about 0.001 M; dotted lines indicate uncertain values)

A. Quinone	G. 1,4-Naphthoquinone	S. $\text{K}_2\text{Mo}(\text{CN})_8$
B. 2-Naphthoquinone	H. Indigo tetrasulfonate	T. $\text{Fe}^{+++} - \text{Fe}^{++}$
C. 1,2-Naphthoquinone	K. Indigo disulfonate	V. $\text{K}_2\text{Fe}(\text{CN})_6$
D. 1-Naphthol-2- C^+ ion	M. 1,8-Anthraquinone disulfonate	W. $\text{V}^{+++} - \text{V}^{++}$
E. 1-Naphthol-2- C^+ ion	N. 9-Anthraquinone sulfonate	X. $\text{Na}_2\text{S}_2\text{O}_8$
F. Methylene blue	R. $\text{Ti}^{+++} - \text{Ti}^{++}$	Y. $\text{Cr}^{+++} - \text{Cr}^{++}$

reducing system itself whose potential would be the "apparent reduction potential." It can be shown on the basis of the equations developed later in this paper, that a difference of 90 millivolts in the potential of two reagents would correspond to differences in rate of the irreversible step of about 1000 fold. A glance at Figure 1 will show that only in certain ranges and at certain pH values can we find, at present, reagents nearer together than 100 millivolts so that we are usually concerned

TABLE I
Apparent reduction potential of certain azo-dyes in 0.2N HCl at 25°

A.R.P. (NORMAL HYDROGEN ELECTRODE = 0)

	volts
	+0.42
	+0.36
	+0.32
	+0.29

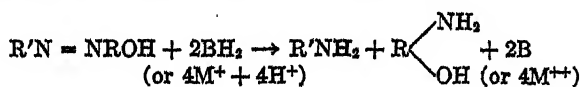
(S = SO₃H group.)

The results are significant within 10 millivolts.

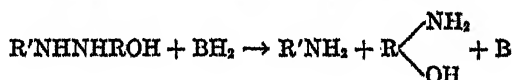
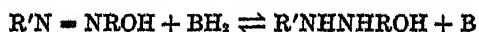
with distinguishing between reactions that are so slow as to be inappreciable and those that take place rapidly; an exact definition of "appreciable reduction" is at present not important.

The relationship between A.R.P. and structure is of considerable interest both practically and theoretically. When sufficient data along these lines have been collected such relationships should replace our present qualitative statements about the relative ease of reduction. The results given in table 1 are

representative of those obtained in a study of the reduction of some 30 azo dyes (13). The determinations were made for the most part in dilute acid solution (pH 0.76) but a number of experiments were also made with various buffer solutions to determine the change of A.R.P. with change in pH. The reaction takes place according to the equation:



It is extremely probable that the reversible step which controls the rate is the formation of the hydrazo compound which then undergoes further reduction with cleavage of the molecule, the last step being irreversible.



The difficulty of isolating ortho or para hydroxy and amino hydrazo compounds accords with this mechanism.

The influence of changes in structure on the A.R.P. is very well illustrated by a series of unsaturated 1,4 diketones and related substances (table 2) measured in acid solution containing a large percentage of acetone to render the compounds soluble (11). Here a slightly different series of reversible systems must be employed and the experimental difficulties are considerable. There is also a question what standard of potential should be chosen with such a solvent but the results are comparable with those in alcoholic and aqueous solution within 40 to 60 millivolts as measurements of the A.R.P. of benzoylacrylic acid in all three solvents have shown. A discussion of the significance of these results from the standpoint of the organic chemist is hardly justifiable in a general review of this sort, but a matter of some importance is involved in a comparison of the unsaturated 1,4 diketones and quinones, both of which contain the conjugated diketonic system $\begin{array}{c} \text{---C---C=C---C---} \\ \parallel \qquad \qquad \parallel \\ \text{O} \qquad \qquad \text{O} \end{array}$. If it be as-

sumed that the addition of hydrogen or removal of hydrogen with this particular system involves a 1,6 addition or elimination process, the reversible behavior of quinone on the one hand and the irreversibility of the diketones on the other is readily understood (11, 8). In the one case the di-enol which can be "dehydrogenated" being stable is always present in solution, in the other it has only a transient existence and is irreversibly

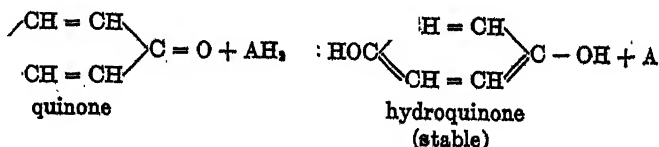
TABLE 2
Apparent reduction potentials

SUBSTANCE	SOLVENT	APP. RED. POT. (NORMAL HYDROGEN ELECTRODE = 0)
(A) Certain unsaturated compounds		
Dibenzoylethylene (cis and trans) ($C_6H_5COCH=CHCOOC_6H_5$).....	a	+0.27 (± 0.02)
Benzoylacrylic acid ($C_6H_5COCH=CHCOOH$).....	a	+0.06 (± 0.04)
Benzoylacrylic acid.....	b	+0.06 (± 0.04)
Benzoylacrylic ester ($C_6H_5COCH=CHCOOC_2H_5$)...	a	+0.06 (± 0.04)
Maleic ester ($C_6H_5OOCCH=CHCOOC_2H_5$).....	a	-0.25 (± 0.06)
Maleic acid.....	a	-0.25 (± 0.06)
Maleic acid.....	b	-0.25 (± 0.06)
(B) Certain nitro compounds		
1, 3, 5 Trinitrobenzene.....	a	+0.26 (± 0.02)
2, 4 Dinitrobenzoic acid.....	b	+0.23 (± 0.02)
1, 3 Dinitrobenzene.....	a	+0.16 (± 0.01)
m-Nitrobenzoic acid.....	b	+0.06 (± 0.04)
Nitrobenzene.....	a	+0.06 (± 0.04)
Phenylnitromethane.....	a	-0.08 (± 0.06)

a = 75 per cent acetone, 25 per cent aqueous hydrochloric acid; total acidity 0.2N.

b = aqueous 0.2N HCl.

isomerized to the diketone; such an explanation is in harmony with our general knowledge of the keto-enol changes in such systems.



potential called the "normal" potential (E'_o).³ At a definite hydrogen ion concentration the equation relating the potential of a reversible system (E_h) and the concentration of the components at 25° is as follows:

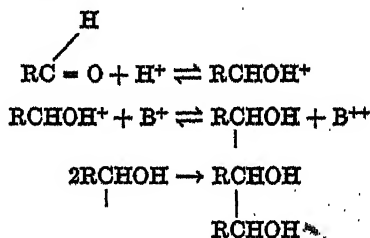
$$E_h = E'_o - \frac{0.059}{n} \log \frac{[\text{Red.}]}{[\text{Oxid.}]}$$

where n is the number of hydrogen equivalents involved in the process; as a rule with organic systems $n = 2$, with the inorganic ions $n = 1$. It is readily calculated that if a "pure" reducing agent contains 1 per cent of the oxidized form its potential will be $2 \left(\frac{0.059}{n} \right)$ volts greater than the normal (i.e., equimolecular) potential. What a reagent will accomplish by being oxidized from the "pure" state to 1 per cent oxidized is not very important so that we may consider the "working range" of a reagent to be its "normal" potential ± 0.059 volts if $n = 2$, or ± 0.118 if $n = 1$. It is to be noted that this "normal" potential must be taken as that of the equimolecular mixture under the conditions specified. The calculation of this from one set of experimental conditions to another is unfortunately complicated by the so-called "salt effect," the formation of complex ions, and the change in acidity of the solution. An exact treatment takes one far into the question of the difference between concentration and activity, evaluation of liquid junction potentials and other recondite electrochemical matters. The complications are not so great with most organic systems as they are where we are dealing with those inorganic salts, the activity of whose ions may vary tremendously with changes in concentration and solvent and whose "normal potential" may be more or less in the nature of an abstraction. If we confine our attention to those systems in which the activity coefficient of both components is not very different from unity and is affected to the same degree by slight changes in solvent, we are on relatively

³The European convention in regard to sign has been used throughout this paper; the potential of the chlorine electrode has been regarded as positive, that of the alkali metals as negative.

safe ground. In applying electrochemical ideas to a specific problem, one must be ever on one's guard that the simple equations involving concentrations and not activities are sufficiently close approximations for the purposes at hand. A test of this point may be obtained by studying the variations in potential with changes in concentration and solvent. It is difficulties of this sort, more than anything else, which limit the practical usefulness of the electrochemical ideas. However, the reagents listed in figure 1 are those which, *in the pH range plotted and in dilute solution* are generally free from large "salt effects" and are reliable enough for the purposes at hand.

If the irreversible step is a monomolecular reaction, then the "apparent reduction potential" will be essentially independent of the total concentration; this has been shown to be the case with the azo compounds and the 1,4 diketones, and is probably true in many other instances. Where this is not the case the situation is more complicated and the "apparent reduction potential" is a less satisfactory concept, necessarily varying with the concentration. The reduction of the "halochromic salts" of aryl carbinols, unsaturated ketones and certain aldehydes afford examples of this sort (9). Here the rate of the irreversible step which is bimolecular is a function of the total concentration; nevertheless even here one can state with some assurance that only reagents of less than a certain potential will bring about the reaction under definite conditions. The bimolecular reduction of an aldehyde by a homogeneous reducing agent (B^+) is illustrated below:



The temperature would be expected to affect the speed of the irreversible reduction but its effect on the "apparent reduc-

tion potential" can not be predicted in general as several different factors are involved. In the case of those compounds so far studied, a change of 50° has not very greatly affected the values which would be assigned to the A.R.P. (13).

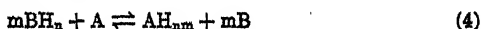
In general, one can predict that the calculated quantity of the "pure" reducing agent should bring about rapid and complete irreversible reduction (under the conditions specified) if the normal potential is more than about 60 millivolts lower than the A.R.P. of the compound; if more than 100 millivolts higher the reagent will be "without action." A reagent falling between these ranges might be expected to cause slow reduction, the rate of which would decrease as the reaction proceeded for reasons given below.

With the exception of stannous chloride and sulfurous acid, most of the usual soluble reducing agents are shown in figure 1. A consideration of the potentials there plotted and the A.R.P.s given in table 1 and 2 lead to many obvious predictions to which as far as I am aware, there are no exceptions. The difficulties of the stannous-stannic system are of the type mentioned above and its potential is difficult to measure. In fairly concentrated hydrochloric acid an equimolecular mixture of stannous and stannic chlorides has a potential of $+0.4$ (20). Predictions based on this value *under precisely these conditions* will not be greatly in error.

The case of sulfurous acid is different from the other soluble reducing agents commonly employed; its oxidation to sulfuric acid is irreversible, and a study of it presents just the reverse of the problem we have been considering. No real oxidation-reduction potential can be given but an approximate "apparent oxidation potential" might be evaluated by methods to be discussed in a later section. One would expect that if the A.R.P. of a compound were about that of the "apparent oxidation potential" of sulfur dioxide under the same conditions, reaction would occur. The general information about the uses of sulfur dioxide as a reducing agent is in accord with this idea, but no special study has been made as yet.

The derivation of certain approximate equations

For the sake of completeness, the following simple algebraic analysis of the factors concerned in the preceding discussion is given; the discussion is based on concentrations throughout for simplicity's sake and because the requisite information in regard to activities is in most cases lacking. Where organic reversible systems are concerned the equations may be expected to hold fairly well, with inorganic salts for the reasons given above they are more uncertain. Since, according to our theory, it is the amount of material present as a result of the reversible reaction which governs the speed of the entire process, these equations are useful in making approximate predictions in regard to the effect of the relative amount of reducing agent and other variables. If we represent the reversible step by a general equation where AH_{nm} is the



material undergoing the irreversible transformation we obtain the following equations:

$$(E'_o)_A (\text{pot. } A/AH_{nm}) - (E'_o)_B (\text{pot. } B/BH_n) = \frac{0.059}{nm} \log \frac{[AH_{nm}][B]^m}{[BH_n]^m[A]} \quad (5)$$

(reversible step) (reducing mixture)

If we start with z moles of reducing agent (BH_n), y moles of its oxidized form and c moles of A per liter and let x be the fraction of A converted to AH_{nm} as a result of the reversible step we have:

$$\text{Diff. in potential} = (E'_o)_A - (E'_o)_B = \frac{0.059}{nm} \log \frac{cx(y + mcx)^m}{(z - mcx)^m c (1 - x)} \quad (6)$$

If cx is small compared to c , z and y , we can write approximately:

$$(E'_o)_A - (E'_o)_B = \frac{0.059}{nm} \log x + \frac{0.059}{n} \log y - \frac{0.059}{n} \log z \quad (7)$$

If $z = y$ in equation 7 we have the simplified approximate equation:

$$(E'_o)_A - (E'_o)_B = \frac{0.059}{nm} \log x \quad (8)$$

The concentration of the substance undergoing the irreversible change is $x(c-s)$ where c is the initial concentration of the substance A and s is the amount of A which has been irreversibly reduced at any given time. We may, therefore, write the usual equation for a monomolecular reaction:

$$\frac{ds}{dt} = kx(c-s) \quad (9)$$

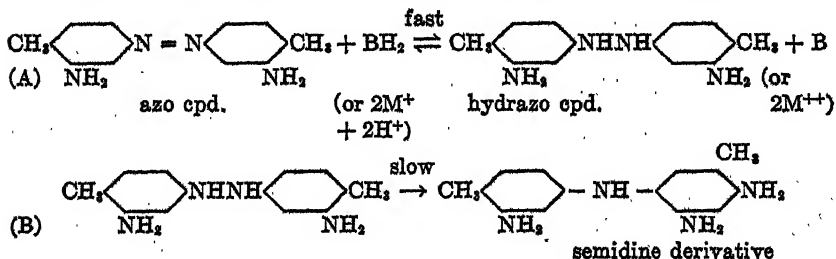
If a large excess of reducing agent containing an equal amount of the oxidized form is employed, x will be essentially constant throughout the entire reaction and we may integrate equation 9 obtaining equation 10 in which the usual constant k_1 is equal to kx . It is obvious that for a given transformation k is constant but x may be varied by using reducing agents of different potential: therefore the rate of the entire process (k_1) will be a function of the potential of the reagent employed.

$$k_1 = kx = \frac{2.303}{t} \log \frac{1}{1-s} \quad (10)$$

If we use about an equivalent quantity of "pure" reducing agent (y small but not zero) a more complicated situation results; if $c = z$ and cx is small compared to y and c , equation 7 still holds approximately, but x is not constant during the course of the reaction. As the reaction proceeds z becomes smaller, y increases; these changes will continuously decrease the value of x and thus the rate of the irreversible step ($k_1 = kx$). If we start with one equivalent of reducing agent containing 1 per cent of oxidized compound, the value of x will fall throughout the course of the reaction from its initial value as follows ($n = 2, m = 1$); 10 per cent reduction 1/10; 50 per cent, 1/100; 90 per cent, 1/1000. The decrease in speed of the reaction will correspond to these variations in the amount of labile reduction product formed by the continually readjusting equilibrium reaction. Between 10 and 90 per cent reduction, the rate will vary ten fold either side of that which is characteristic of the half-completed reaction. With larger amounts of oxidized form present in the reducing mixture this variation becomes less, until with an equimolecular mixture

(such as that used in the method of determining A.R.P.), the rate is nearly constant for the first half of the reaction and has only diminished by about one tenth at 90 per cent reduction. In other words, the relatively rapid changes brought about with the nearly "pure" reducing agent for the first half of the process have disappeared. Such complications affect to some extent criteria as to reduction or non-reduction based on color change of "pure" reagents since it is often difficult to say at what concentration the color of the oxidized or reduced reagent becomes imperceptible. However, if one does not attempt too fine distinctions, the predictions based on "apparent reduction potentials" and the normal potentials of the reagents employed can be applied to the use of "pure" reducing agents since even a thousand fold variation in rate corresponds to only 90 millivolts difference in the potential of two different reagents (if $\text{nm} = 2$).

Büllmann's interesting electrochemical study of certain azo compounds (1) affords an excellent example of a reversible reduction followed by an irreversible reaction and in this instance it is possible to study both processes experimentally. Certain derivatives of azobenzene form with the corresponding hydrazo compounds a strictly reversible system in dilute aqueous acid. The system is not stable; however, as the hydrazo compound in the acid solution rearranges irreversibly to a semidine derivative (eq. B). The speed of this rearrangement has been determined by noting the rate of change of potential of the azo-hydrazo system; the initial potential was calculated by an extrapolation to zero time. Since the intermediate compound is relatively stable, we are in a fortunate position with regard to this particular system, and can measure both the potential of the reversible system and the speed of the irreversible step.



Let us suppose that we were to treat the azo compound in dilute acid with a large excess of a reducing agent (BH_2) containing an equal amount of its oxidation product (B) (or an inorganic positive ion M^+ and its oxidized state M^{++}). The reversible reaction would proceed to a definite equilibrium (eq. A) the composition of which can be calculated from the known potential of the azo-hydrazo system (0.373 at 18° in 0.1 N HCl) and that of the reducing system ($\text{BH}_2 - \text{B}$, or $\text{M}^+ - \text{M}^{++}$). Since we have specified a large excess of a reducing agent containing its oxidation product, equation (8) above serves to calculate the fraction x of azo compound converted to hydrazo as a result of the reversible step and this fraction will be practically constant throughout the process. Biilmann has found the rate of the rearrangement process at 18° in the presence of acid to be such that $k = 0.076$ (half life of nine minutes). Substituting this value and that of x calculated from equation (8) into equation (10) we can predict the rate of semidine formation with any reducing agent we may choose to employ at 18° , always assuming that there are no disturbing influences on the rate of the irreversible step. The calculations are given below for a few hypothetical reducing agents of different potential: (E'_0 for the azo-hydrazo system being +0.373)

E'_0 OF REDUCING AGENT	x CALCULATED FROM Eq. 8 ($n = 2$)	$k_1 = kx = 0.076x$	TIME FOR 20 PER CENT OF REACTION CALCULATED FROM EQ. 10
			minutes
+0.402	0.1	0.0076	81
+0.431	0.01	0.00076	810
+0.460	0.001	0.000076	8100

It is evident that with the first reducing agent (+0.402) we would get an appreciable reduction in half an hour but with the other two it would take five and fifty hours respectively to reduce 20 per cent of the azo compound to the semidine; certainly in the last case evidence of reaction would be detected only by the most patient investigator! Reagents of lower potential than 0.402 (more powerful reducing agents) would cause still more rapid reduction, the maximum rate possible being that corre-

sponding to the rearrangement of the pure hydrazo compound (20 per cent in three minutes).

Here, then, is a very definite example of an irreversible transformation conditioned by the potential of the reducing agent employed. It should be noted, however, that this state of affairs would be true only if the reducing agent and the azo compound *react rapidly in comparison with the rate of the irreversible step*. If the speed of the irreversible step is faster than the speed of attaining equilibrium, then no equilibrium will be reached and the potential of the reagent employed will be of no significance. Since at 18° the formation of semidine is rather slow and the reaction of the azo compounds and most soluble reducing agents rapid, our predictions in this instance seem likely of experimental verification.

Catalytic hydrogenation and heterogeneous reduction

If a solution of one component of a reversible system is shaken with hydrogen and a catalyst (platinum or palladium, for example) an equilibrium will be established which can be predicted from the normal potential of the reversible system and the potential of the hydrogen electrode in the solvent in question (Denham (15)). This is merely a special case of two reversible systems interacting, the catalyst seeming to make the system H_2 gas/ H^+ truly reversible. If a compound whose irreversible reduction can be formulated in terms of reduction potentials is similarly treated, reduction will occur if the "apparent reduction potential" is somewhat above that of the hydrogen electrode. The hydrogen-platinum catalyst system is here functioning like any other reversible reducing agent, and reduction is presumably proceeding through the same two steps which are characteristic of the reactions hitherto discussed. However, if the catalyst-hydrogen combination is able to add hydrogen atoms to the compound by some mechanism *not involving a reversible step*, then the presumption is that the process may proceed although the "apparent reduction potential" is below that of the hydrogen electrode. Such a case has been experimentally examined in some detail (8). Of the known soluble reducing agents which

can be employed in aqueous solution *only* chromous chloride ($E' = -0.40$ at $\text{pH} = 1$) reduces maleic acid, yet hydrogen and a suitable catalyst ($E' = -0.059$ at $\text{pH} = 1$) hydrogenates the substance fairly rapidly. A large number of ethylene derivatives, which are not reduced even by chromous chloride, can be "catalytically hydrogenated" in solution at room temperature. Here plainly the process of catalytic hydrogenation is running a special course, which is in no sense a function of the potential of this reagent. Such hydrogenations or reductions, can not be formulated in electrochemical terms and probably involve the direct irreversible addition of hydrogen to the ethylene linkage. Catalytic hydrogenation is an excellent illustration of a process of reduction which as a rule can not be treated from the electrochemical point of view.

The use of two phase systems in bringing about reduction is very common, but very difficult to treat quantitatively. As the pioneer researches of Haber on reduction at a cathode showed, the rate of diffusion to the active surface is of great importance and may be the governing factor. The subject of cathodic reduction is amply treated, both from the practical and theoretical standpoint in texts on electrochemistry, it would be unwise to attempt to add to the discussion beyond calling attention to the fact that cathodic reduction may involve a reversible step or may like catalytic hydrogenation proceed directly and irreversibly. If the difficulties of diffusion rates could be eliminated or better controlled, these two possibilities might be investigated more fully in a number of instances; the results perhaps could then be correlated with the action of soluble reducing agents.

Much the same difficulties are encountered when one attempts to deal with the action of metals on solutions of organic compounds. To assign a definite potential to a metal against a solution of its ions is easy, but to say whether this potential actually controls the process of irreversible reduction is at present impossible. From the more or less qualitative evidence familiar to all organic chemists, it seems as if the reducing action of metals were a function of their potential. But more than

this can not now be stated. As in the case of catalytic hydrogenation and probably cathodic reduction, the possibility always must be considered that some special irreversible process is taking place that is in no direct way connected with the reducing potential of the metal.

OXIDATION AND DEHYDROGENATION

Of the great variety of transformations embraced by the term oxidation, only those which take place in solution at ordinary temperatures come within the scope of this review; how many of these can be formulated electrochemically is a matter for future experimentation to decide. At present it is possible only to indicate certain probable lines of attack and to suggest certain general principles which would seem to apply as surely here as in the much simpler field of reduction.

The reversible processes present little difficulty. The oxidation of hydroquinone, or of leuco indigosulfonate in solution by a component of another reversible system proceeds to a final equilibrium which can be as definitely predicted as any process in organic chemistry. The rate at which such equilibria are established when one is dealing with the usual soluble oxidizing agents happens to be very rapid, but concerning it our electrochemical equations give no information. Whether leuco indigosulfonate in a certain solution is "completely" oxidized or not is a question which can be answered satisfactorily in terms of electrochemical potentials if one uses such oxidizing agents as quinones, potassium dichromate or ferricyanide. If some compound not a component of a reversible system is taken as the "oxidizing agent" (e.g., an azo dye) then the problem is simply the one we have been considering under the head of irreversible reduction.

In line with the general theory developed in the introduction to this paper, we should expect to find a relation between the potential of the reagent employed and the rate of the irreversible oxidation only if some reversible step controlled. The oxidation of an ethylenic linkage by ozone (proceeding through the ozonide) or the addition of hypobromous or hypochlorous acid

to an unsaturated compound are specific reactions of these reagents which are obviously not controlled by their oxidizing potential; to attempt to connect these reactions with potentials would probably not occur to anyone. The same may probably be said of the addition reactions of the halogens which though formally an "oxidation" are often considered by organic chemists as closely akin to the addition of the halogen acids or "nascent" hydrogen. We seem here to be concerned with the addition of specific things to a non-polar molecule and are not dealing with

TABLE 3

Determination of A.O.P. of 1,2- and 1,4-Aminonaphthol in 0.2 N HCl at 25°

OXIDIZING REAGENT (CONTAINING EQUIMOLECULAR AMOUNT OF REDUCED FORM)	NORMAL POTENTIAL IN pH 0.76 (NORMAL HYDROGEN ELECTRODE = 0) OF REAGENT	CHANGE IN POTENTIAL (MILLIVOLTS) (ON ADDING HYDROCHLORIDE OF AMINOPHENOL)			
		1,4 aminophenol		1,2 aminophenol	
		5 minutes	30 minutes	5 minutes	30 minutes
Indigo tetrasulfonate.....	+0.326	0	0	0	0
1,4 naphthoquinone.....	+0.426	0	0	0	0
2,6 dimethoxyquinone.....	+0.471	3	12	0	0
1,2 naphthoquinone.....	+0.506	17	50	7	14
2,5 dimethylquinone.....	+0.449	70	95	38	43
Potassium ferrieyanide.....	+0.631	170	185	126	132
Quinone.....	+0.656	185		136	
Ferric chloride.....	+0.726	250	280	220	228
K ₂ Mo(CN) ₆	+0.776	325	332	270	280

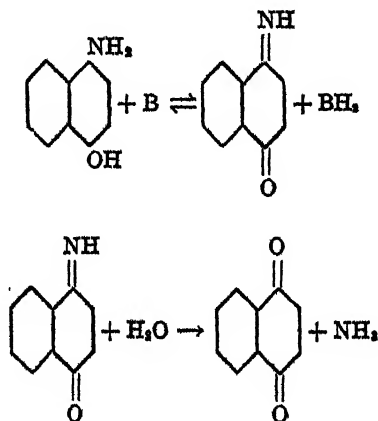
From the above, the A. O. P. in pH 0.76 at 25° of 1,4 aminonaphthol is +0.460 \pm 0.010 millivolts; of 1,2 Aminonaphthol is +0.500 \pm 0.010 millivolts.

a series of more or less "powerful" reagents capable of bringing about the same change.

The irreversible changes which involve the elimination of hydrogen and which can be brought about in solution at room temperature are the most likely subjects for an electrochemical investigation of the type we are here discussing. To what extent are the rates of such reactions conditioned by the potential of the oxidizing agent employed?

The oxidation of the aminonaphthols to the corresponding

quinones (which is very rapid in acid solution at room temperature) has been investigated by means of a series of reversible oxidizing agents and an electrochemical device for following the reaction identical to that used in studying reductions (13). An "apparent oxidation potential" for these substances can be given with assurance as table 3 shows. The irreversible step is here undoubtedly controlled by the formation of an easily hydrolyzed quinone imine.

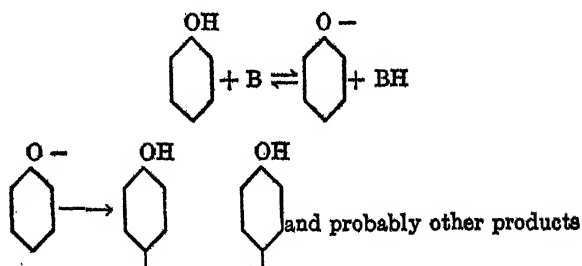


With this particular transformation, the potential of the reagent controls.

The oxidation of benzidine and certain other diamines to highly colored meroquinones has very recently been studied (Clark (5)) and found strictly reversible. These compounds, however, are very unstable in many solutions and pass irreversibly into other products. Such an irreversible transformation (under conditions where its rate is slower than that of the initial reversible oxidation) should be also capable of formulation in terms of "apparent oxidation potentials."

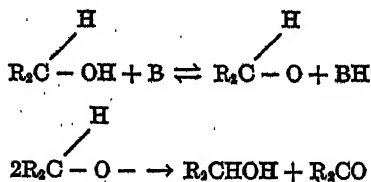
The researches of Goldschmidt (22) and Pummerer (30) have shown that in the oxidation of certain phenols the initial formation of a free radical can be demonstrated. It is probable that a similar process is involved in most irreversible oxidations of phenolic substances and if such a reaction is reversible it may

well control the rate of a subsequent irreversible rearrangement, as illustrated by the following equations:



The fact that it has been experimentally demonstrated that the formation of a free radical by reduction is a reversible reaction governed by the usual electrochemical laws (14) suggests that the formation of a monovalent oxygen radical by oxidation would also be reversible. Preliminary investigations have shown that the oxidation of many phenolic and enolic substances is dependent on the potential of the oxidizing agent employed (13). Similar considerations very probably apply to the oxidation of many amino compounds to hydrazines and mercaptans to disulfides. Considerable work has been done on the mechanism of amine oxidation from a strictly chemical viewpoint which would point to such a formulation as that given above (22a).

One is tempted to extend this mechanism to the oxidation of alcohols, hydroxy acids and aldehydes (in the form of their hydrates according to the Wieland concept (44)) and imagine that a second irreversible step consists of a "disproportionation" of the "odd molecule." Although such an extension is at present pure speculation, I venture to include it in this review as



being in the nature of a working hypothesis which may lead to interesting experiments.

It is important to establish whether or not the oxidation of such hydroxy compounds (which in contrast to the oxidation of the phenols may be called monomolecular oxidation) is governed by the potential of the reagent employed. For example, will the action of Fehling's solution or ammoniacal silver solutions on aldehydes be duplicated by any reagent of an equally high oxidizing potential at the same alkalinity? Is the oxidation of an aqueous solution of acetaldehyde by quinone in the presence of palladium (Wieland (43)) a specific action of quinone and closely related substances or does it merely depend on having a "powerful" enough oxidizing agent? What relation exists between the rate of oxidation processes brought about by palladium (43), platinum (2, 3), charcoal (41, 44), or ferments (see below) and the potential of the oxidizing agents employed? Similar questions can be raised in regard to the oxidation of unsaturated compounds with permanganate. Now that our knowledge of the potential of the reversible systems has become greater, we can look forward to obtaining an answer to these questions in the near future. In advance of such answers, we must be cautious in attempting to apply the electrochemical point of view to these reactions.

The work of Wieland during the last fifteen years (44) has strikingly demonstrated that many of our oxidation reactions are not concerned with the addition of oxygen but with the removal of hydrogen atoms,—“dehydrogenation” as he has called the process. The electrochemical study of reversible systems is entirely in accord with this viewpoint and, indeed, throughout this review I have used the word oxidation as almost equivalent to dehydrogenation. This separation of our ideas concerning oxidation from any idea of oxygen transfer is of the greatest importance and Wieland's work has done much to counteract the over-emphasis placed on oxygen as an oxidizing agent. Since the electrochemical point of view starts from the chemistry of ions capable of changing their valence by the gain or loss of one or more electrons, the term oxidation has never suffered in this branch of science from too close attachment to the element oxygen. I have preferred to use the term oxidation throughout

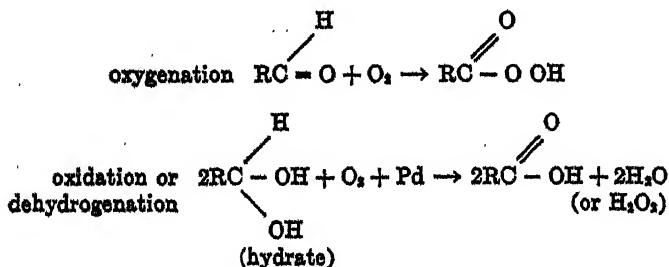
rather than dehydrogenation as the former term has become irrevocably attached to the change of ferrous to ferric salt and of ferrocyanide to ferricyanide. Although the latter process can be thought of as a loss of hydrogen when one considers the corresponding acids, the change of valence of positive ions is hard to fit into the "dehydrogenation" terminology. The same may be said of the oxidation of the triphenylmethyl ion to triphenylmethyl itself (hexaphenylethane) (14). The most satisfactory definition of oxidation in the electrochemical sense would seem to include the process of dehydrogenation, and the removal of electrons from positive and negative ions. At least we know examples of all three processes in organic chemistry which can be measured electrochemically. Personally, I am of the opinion that the *reversible* dehydrogenations are probably only special cases of reversible electron transfer, but until this point can be better established and we have more information concerning *irreversible* dehydrogenation it would seem to be unwise to use the electron picture too freely.

Oxygen as an oxidizing agent

Oxygen in the absence of a catalyst is not a component of a reversible oxidation-reduction system. Even in the presence of platinum and other catalysts it is very doubtful if a real equilibrium is reached involving oxygen and its reduction products (water or hydrogen peroxide or the hydroxyl ion). The potential of the hypothetical oxygen electrode can be estimated, however, indirectly; this potential would be characteristic of the behavior of oxygen only in the presence of a catalyst which functioned as surely as platinum functions with hydrogen. The reactions of oxygen under ordinary conditions are in no way connected with this more or less mythical potential.

A glance at figure 1 demonstrates that it is only because of this electrochemical inertness of oxygen that we are able to handle solutions of hydroquinone and similar substances in air without appreciable oxidation. Yet oxygen combines extraordinarily rapidly with free radicals containing trivalent carbon or with hemoglobin and more slowly with many aldehydes and other

compounds which are "auto-oxidized." In the case of hemoglobin the combination is strictly reversible and parallel to the combination of this remarkable protein with carbon monoxide or nitrous oxide; the process has nothing in common with the oxidation of hemoglobin to methemoglobin by oxidizing agents (7,10). The term "oxygenation" might be used to distinguish this addition of molecular oxygen which is more nearly comparable to the addition of ammonia to complex inorganic amines than to oxidation processes. Similarly, one is tempted to think of the formation of peroxides from free radicals and the "auto-oxidation" of numerous substances as being essentially oxygenation: the terminology is here a difficult matter, as few people would care to give up the term "auto-oxidation." Wieland uses the term "oxidation" much as I have used "oxygenation," and the term "dehydrogenation" for oxidation, as explained above. His study of the action of oxygen on dry and moist aldehydes in the presence of palladium (44 IV) seems to show that oxygen can attack a moist aldehyde in the presence of palladium in the two ways which are illustrated by the following equations:



The first reaction written above, whether it be called an auto-oxidation or oxygenation, has no more to do with oxidation potentials than the addition of hydrogen chloride to ammonia or to pinene. The many interesting results which have been obtained in the last few years in connection with "negative catalysts" for this reaction (28, 35) must therefore be omitted from this review. Whether the second reaction can be handled from the standpoint of electrochemistry is a problem closely akin to that raised earlier in this discussion in connection with the

action of quinone and palladium on aldehydes. Until such irreversible oxidations have been more thoroughly studied with a variety of oxidizing agents of definite potential a formulation of the action of oxygen is evidently impracticable.

The reduction of molecular oxygen by soluble reducing agents would seem to lend itself to an electrochemical inquiry. This process is usually attended by the formation of hydrogen peroxide or its salts. This is true both of the oxidation of solutions of many inorganic salts and also of alkaline solutions of phenols and hydroquinones (24). It appears that the rate of reduction of oxygen by a component of a reversible system (e.g., a hydroquinone or ferrous salt) is not a function of the potential of the system but of certain specific properties of the various reagents (17, 24). The fact that in alkaline solution an *anthra*-hydroquinone sulfonic acid reacts with oxygen more rapidly than does the more powerful reducing agent, sodium hyposulfite (Fieser (18)), is only one instance of this sort. Without attempting to review the enormous literature dealing with auto-oxidation in aqueous solution, it seems evident that a prediction as to the rate of oxygen absorption based on the potential would be very uncertain. Moreover, oxidations by atmospheric oxygen are very susceptible to homogeneous as well as heterogeneous catalysis, and an accurate statement as to the rate of oxidation by oxygen of a really pure substance would have to be made with caution.

Certain biochemical problems

Since the interaction of oxygen with reversible systems probably can not be treated electrochemically, there seems but faint hope of applying the methods to the irreversible oxidations (dehydrogenations) brought about by this gas in biochemical processes. Here again catalysis both homogeneous and heterogeneous plays an enormous rôle. Warburg (39, 40, 41) and Meyerhof (27, p. 16-22) have shown the startling effects of small amounts of metallic salts in promoting and small amounts of cyanide in retarding oxidations with atmospheric oxygen both in the living cell and in vitro (45). The biochemical oxidations

brought about by methylene blue, unlike those with oxygen, do not seem to be susceptible to this anti-catalytic influence of cyanide if we may judge from the experiments of Szent-Györgyi (34) and Fleisch (19) with muscle tissue and sodium succinate. The former investigator has developed the thesis that in biochemical processes there are two distinct types of catalysts at work,—one activating the oxygen, the other bringing about the oxidation (dehydrogenation) of the organic material. In Warburg's opinion (39, 40) complex iron compounds are the oxygen catalysts which function by virtue of an oxidation to the ferric state which is then reduced to the ferrous by the organic compound being burned in the living cell. Biochemically methylene blue is thus the equivalent of oxygen plus an iron catalyst. This separation of the problem of biochemical oxidation into two separate phases seems to be justified by all the facts now available and clears away much of the controversy between those who insisted on oxygen activation and those who laid emphasis on dehydrogenation (42, 36, 40).

The second aspect of the problem,—the so-called activation of the organic molecule—is closely allied to the action of metals, metallic colloids, and charcoal, in promoting the oxidation of organic compounds by quinone, methylene blue, etc. The living cell contains something that acts in a similar manner. (5, 25, 26, 27, 37). The possibility of attacking this problem of "catalytic irreversible oxidation" by the use of a series of reversible systems, I have already mentioned. Biochemical catalysis would seem to form merely one branch of this general subject.

In connection with Warburg's theory, Spoehr's recent discussion (33) of the catalytic influence of iron phosphate on the oxidation of sugars is of interest as bringing out clearly two distinct mechanisms by which oxygen might be activated. He considers that in the case of his iron complex salt, oxygen may be either directly taken up (much as it is by hemoglobin) or may change the iron from the ferrous state to the ferric. According to the first hypothesis, the "oxygenated compound" would interact directly with the sugar; according to the second, the ferric

salt would be the oxidizing reagent and in turn would be reduced. If the latter mechanism is correct the oxidation-reduction potential of the complex may be of significance as Spoehr points out. If reversible oxidation-reduction systems of this type are present in nature, it is very probable that their behavior may be formulated ultimately in electrochemical terms. W. Palladin (29) has suggested a quinone-like system functioning in plants. Warburg's theory of iron complexes has been mentioned above and glutathione (isolated from tissue by Hopkins (23)) has been viewed as a reversible system aiding oxidation. Meyerhof (27, p. 35), however, believes that the simple change of the $-SH$ group to the $-S-S-$ involved in the oxidation and reduction of glutathione and cystine is not sufficient to account for its function in the living cell; his experiments indicate that some sort of a peroxide must be involved. The oxidation of such systems by oxygen seems to be subject to metallic salt catalysis (30).

It is uncertain whether or not the cystine-cystein and the glutathione system are reversible and can be formulated in electrochemical terms. Dixon (16) by using carefully prepared electrodes obtained reproducible potentials with solutions of cystine and cystein and glutathione and the corresponding disulfide, but the potentials were independent of the relative concentration of the two components of the system. Since dependence on relative concentrations is the most important criterion of reversibility one is inclined to believe that his potentials are not characteristic of the behavior of these systems. He suggests that we are here dealing with a new type of electrode system but the problem remains as to how one should interpret the potential of such a system. In this connection it should be mentioned that carefully prepared electrodes will often record fairly reproducible potentials in pure buffer solutions in the absence of any oxidation-reduction systems; the e.m.f. varies regularly with the hydrogen ion concentration. It is possible that some such "accidental" potentials were the source of the electromotive force measured in Dixon's work. A further study of disulfide systems using a series of reversible reagents of known potential seems advisable.

Evidence for the existence of reversible systems in living cells is afforded by recent studies from the Hygienic Laboratory (5). Dr. Clark and his coworkers have found that washed bacteria or yeast cells slowly develop the property of reducing a number of the colored oxidation-reduction indicators; the ability to reduce those of higher potential is first manifested. This development of reducing action is accelerated by the addition of a number of soluble substances such as sodium succinate; some water soluble substance seems to be formed in the cell which is oxidized by the dye. That this unknown substance is a component of a reversible system is strongly indicated by the fact that an inert electrode immersed in these same suspensions develops a fairly definite potential whose magnitude is commensurate with the behavior of the oxidation-reduction indicators. Further work in this direction will be awaited with interest.

Reference has already been made to the oxidation of succinic acid by methylene blue in the presence of muscle tissue. The work of the last few years indicates quite clearly that in the presence of finely divided muscle (Thunberg (37, 38)) or suspensions of certain bacteria (Quastel (31)), equilibrium is slowly attained in the following system at pH values near 7.



Quastel (31) estimating the extent of reduction colorimetrically finds the value for the equilibrium constant K (eq. 11) at pH 7.3 and 45° as 3.0; the value is independent of the amount of "rest-

$$\frac{(\text{fumaric acid})(\text{leuco methylene blue})}{(\text{succinic acid})(\text{methylene blue})} = K \quad (11)$$

ing" bacteria, and the extent of reduction varies with the relative concentrations of the reactants in accord with the mass action law.

Thunberg (37) has recently calculated the potential of the same system in the presence of muscle tissue at pH 6.7 from his observations on the extent of decolorization of methylene blue and Clark's value for the methylene blue potential; his value is +0.005. A value for the same system at pH 7.3 in the presence

of bacteria calculated from Quastel's K and Clark's data is -0.009 . The agreement is good when it is recalled that the change in 0.6 of a pH unit might be expected to lower the potential 36 millivolts (if the reversible process involves only the loss or gain of two hydrogen atoms); furthermore, the temperatures of the two experiments were not identical. The fact that two different investigators using different biochemical catalysts obtain essentially the same equilibrium constant is convincing evidence that the process is strictly reversible under these conditions. The catalytic action in the case of both the bacteria and muscle tissue is probably connected with the two phase system necessarily present but the discovery of any method of changing an irreversible system into a strictly reversible one is of much importance to the general theory of oxidation-reduction reactions quite apart from biochemistry. To what extent this reversible system is involved in other types of biological oxidation, remains to be discovered. The fumaric-succinic acid system by itself can evidently not function as an "oxygen carrier" since, as has been stated above, the action of oxygen on succinic acid seems to be conditioned by some catalyst which is poisoned by hydrocyanic acid.

Criteria of reversibility

In future developments of the concept of oxidation-reduction potentials, both in connection with biochemical and strictly chemical problems, it would seem to be essential to determine at the outset whether a given system is reversible or not. If reproducible electrode potentials are obtained which accord with the logarithmic equation, one can hardly go wrong in accepting the reversibility of the system as established. However, as experience with a number of inorganic systems has demonstrated, there may be difficulties in measuring potentials even of reversible systems; other practical criteria are therefore of service. If one attempts to determine by the methods discussed in this review, the "apparent reduction potential" of the oxidized component and the "apparent oxidation potential" of the reduced form, certain peculiarities will manifest themselves in the case

of reversible systems. In the first place, if the electrochemical method of following the course of the reaction is employed, there will be a rapid initial change of potential when certain reversible systems are used and then no further change; this is in contrast to the behavior of a substance which is irreversibly reduced or oxidized in which case there is a progressive change in potential. In the second place, the particular oxidizing mixture which is appreciably affected by the reduced component, will have a potential *below* that of the mixture which first acts on the oxidized component. In other words, what appears to be the A.R.P. and A.O.P., overlap (actually these terms can only be applied if the processes are irreversible). This is made clearer by the case of a reversible azo-hydrazo system. Thus azobenzenedisulfonate when introduced into a reagent of potential $+0.375$ (containing equal amounts of the oxidized and reduced form) causes an immediate change in potential corresponding to about a 40 per cent reaction; the potential of the same reagent is changed about the same amount, *but in the opposite direction* by the action of the hydrazo compound. Reagents of lower potential reduce the azo compound practically completely; those of higher potential are without action. The normal potential estimated from these results is about $+0.37$; that actually measured by the method of mixtures $+0.38$ (13).

Thus the behavior of both components of a supposedly reversible system towards a series of known reagents gives information in regard to the reversibility; it further enables one to evaluate the potential of the system *if the system is reversible*. It would seem advisable to employ some such procedure in the case of those systems whose potentials have been reported as being independent of the concentrations of the components. The case of glutathione and cystine has already been mentioned; Rideal (21) by the use of specially prepared electrodes ("controlled electrodes") has obtained "irreversible potentials" of a number of sugars in aqueous solution. One is sceptical of the significance of all such potentials until they are checked by some such method as that just outlined which involves the use of reversible systems of known potential.

It may not be out of place to call attention again to the distinction made in the first part of this paper between reversible and irreversible systems. Since the distinction is in the last analysis based on rates of attaining equilibrium there may be some instances where one can draw the line only by means of some arbitrary rule,—these transitional cases (if they exist), however, are unimportant. We find that in aqueous solutions the transfer in both directions of hydrogen atoms or of electrons proceeds with great rapidity with certain compounds,—these are reversible systems and most common soluble reducing and oxidizing agents fall in this category. The equilibrium attained in the interaction of any two such systems might be investigated by sufficiently refined analytical methods (e.g., photochemical) and the results expressed without recourse to electrochemistry. As a general rule, however, these same systems come rapidly to equilibrium with an inert electrode (probably because of an interchange of electrons or possibly hydrogen atoms). Therefore, electrochemistry provides us with a powerful tool for investigating these systems; in order for this method to be effective, electrode equilibrium must be rapidly attained, but this is of importance *only in so far as it is of experimental assistance*. Speed of attaining electrode equilibrium and rapidity of chemical action seem usually to go hand in hand in oxidation-reduction reactions, *but need not necessarily*; the chemical behavior of the components of the system are not conditioned by their interaction with an electrode. Too great an emphasis on the reversibility of electrodes may therefore obscure important results; the testing of an unknown system by means of known reversible systems is therefore of great importance. Whether this experimentation involves electrical or colorimetric methods is of no theoretical importance.

CONCLUSION

Our increasing knowledge of the properties of a number of organic compounds which are rapidly oxidized or reduced in solution has greatly supplemented the list of soluble oxidizing or reducing agents of known potential. By using a graded

series of such reagents, we can decide whether the addition of hydrogen to a given type of compound under definite conditions is reversible or not. If it is an irreversible reaction, we can in many cases determine what reagent is necessary to bring it about; such information is of value in developing a quantitative account of organic reactions and enables us to correlate in such instances structure and chemical behavior. Similarly, a certain number of oxidation or dehydrogenation reactions can be investigated and precise data should be eventually available in this field. It is improbable that the methods will be universally applicable,—indeed, we can be quite confident that that they will only avail when certain mechanisms are concerned; each new type of reaction, each new set of conditions must be experimentally examined. Although the road is thus hedged in by many complications and much arduous work remains ahead, the prospect is alluring to all those interested in the more precise formulation of the fundamental reactions of organic chemistry. It is evident that a consistent quantitative account of the oxidation and reduction of organic compounds will be of great practical value to both organic chemists and biologists. Furthermore, we may feel confident that on such foundation stones a general theory finally will be erected which will bring order out of the present chaos of qualitative information.

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CHEMICAL PROCESSES IN FERMENTATIONS¹

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¹ Revision of a paper prepared to be read at the Los Angeles meeting of the American Chemical Society, August, 1925.—Certain circumstances prevented my arrival in the United States in time to attend the meeting.—The greater part of the German manuscript was translated by Floyd D. Hager.

"It seems to me that perhaps the only advantage of advancing age is that one is able to recall what the old masters thought."—*Graham Lusk*.

1. INTRODUCTION

One of the most important ways in which the products of agriculture are used is the utilization of carbohydrates through their decomposition by the various fermentations, alcoholic, lactic acid, butyric acid, and acetic acid, brought about by micro-organisms. We knew what the original materials and end products are in these biochemical reactions, but we are just in the development of a period whose task it is to penetrate the mechanism of these reactions by use of the tools of biochemistry.

The very great importance and significance of investigations concerning the transformations of carbohydrates and the relation of those transformations to biological science can be indicated by reference to a few facts. The carbohydrates are formed in the assimilation processes of plants and serve as the starting product for the chemical and physical performances of work by the plants. Further, nearly all bacteria take up some form of sugar from their nutrient media. Not only that, but in the animal body carbohydrates can be responsible for the synthesis of protein and fat.

All are familiar with the experiment of two naturalists performed some sixty years ago. They climbed the Faulhorn in Switzerland, a peak 1956 meters high. Seventeen hours before starting they ate the last nitrogenous food taken during the experiment. During eight hours, with one interruption, they climbed, and were in motion a total of thirteen hours. They carefully collected the urine excreted during that time and showed, by its analysis, that the work done corresponded to three times the energy which could be derived from the protein used. This experiment was the starting point of all observations which have shown that, in the case of animals as well as of other more highly developed organisms, the source of energy is not in protein but primarily in fat and carbohydrate.

Workers in this field have shown that the carbohydrates are hereby, in general, burned to carbon dioxide and water. But

the yeasts use for alcohol production, even in case of increased aeration with oxygen, less sugar material than they have at their disposal for this purpose. This shows that in this process there is not only a partial resynthesis of sugar from the intermediate products but from numerous observations made on various organisms, we know that in this disintegration, analogous to purely chemical oxidation, there are intermediate stages.

Excluding, obviously, the case of a biological oxidation, if we are inclined to consider fermentation as a process through which, by a biochemical action carbon chains are broken down or united (see particularly pages 56, 70), it is intelligible that for many decades the efforts of naturalists have been directed toward an interpretation of the decomposition of sugar, using as an example the long known phenomenon of alcoholic fermentation, a process typical of one that stops, to a certain degree, at an intermediate stage.

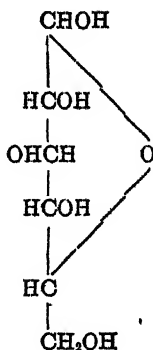
The decomposition of carbohydrate by yeast is expressed, in its quantitative proportions, by the equation of Gay-Lussac:



At first, we are unable to interpret these proportions. The practical quantitative course of the fermentation process produces from each mol of sugar two mols each of alcohol and carbon dioxide. The old familiar formula of glucose,



and the newer one proposed by Haworth for discussion,



give nothing at all of an indication of ethylidene or carbon dioxide groups which occur in the fermentation products. Here, then, is a typical example in which the final condition comes about only through intermediate steps, a process which involves a gradual dissolution of the hexose carbon chain.

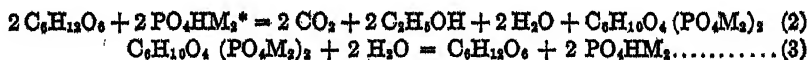
The decomposition of fermentable initial materials in the course of enzymatic alcohol production seems to rest, in the ultimate analysis, on the fact that the substances formed in the course of the reaction are very convertible and difficult to intercept. These substances must, however, in every case, be such that the yeast can further act upon them and render possible in the end the production of compounds which are in equilibrium with each other and which give the end products of the fermentation.

2. THE RÔLE OF ZYMOPHOSPHATES

In regard to the first phase in the decomposition of the sugar complex, i.e., the processes which probably initiate the disintegration of the hexose molecule by the action of the yeast enzyme, to substances containing three carbon atoms, it is difficult to reach any clear cut conclusions from the existing experimental data. It is, however, supposed that in this case the alkali phosphates have an important rôle.

The first proposal, to add to a 15 per cent fermentable sugar solution 0.2 gram per liter of monocalcium phosphate, came from

Pasteur. After Buchner and Hahn had ascribed initial acceleration of fermentation by alkali phosphate to the alkaline reaction of these salts, starting in 1905 L. Iwanow, as well as Harden and Young, began fundamental investigations in connection with this question. The British investigators noted that the speed² of the fermentation increased if one added, in the presence of phosphates, boiled or ultrafiltered yeast juice. This action apparently should be ascribed to the phosphates, as similar observations were made when solutions of the alkali salts of orthophosphoric acid were used. At the end of the fermentation, no phosphate could be detected in the usual way. It must be supposed, therefore, on the basis of later investigations, that in the alcoholic fermentation always two molecules of sugar are concerned, of which one combines with two molecules of phosphate to form hexosediphosphoric acid while the other molecule of sugar breaks up to form carbon dioxide and alcohol. In the further course of the reaction a special enzyme, the phosphatase continually breaks down the hexosediphosphoric acid to inorganic phosphate and fermentable hexose, whereby the repetition of the process becomes possible:



Previously, however, Harden and Young had made, by means of kinetic measurement and analysis, the important observation that the increase in the amount of carbon dioxide or alcohol formed was, within definite limits, in direct proportion to the amount of phosphate added: (see fig. 1). In the graph, curve A shows the normal course of fermentation in the case of yeast juice (l. c., p. 416), curve B the effect of adding phosphate. In the latter case the velocity of fermentation amounted to 9.5 cc. per 5 minutes, i.e., some six times the normal value, and then again reached almost exactly the original value of 1.4. Curve C

² According to Slator (Chem. Soc., 89, 133, (1906)), the speed of fermentation is directly proportional to the quantity of yeast and only very slightly dependent on the sugar concentration between 0.5 and 20 per cent.

*M = metal.

shows the repetition of the whole phenomenon after a period of 70 minutes when the phosphate was renewed.

This organic phosphoric acid compound, which is not precipitated by magnesia mixture, was isolated, as the copper salt, by L. Iwanow in 1905; it can also be precipitated by lead acetate. This Russian investigator considered it a triose derivative hav-

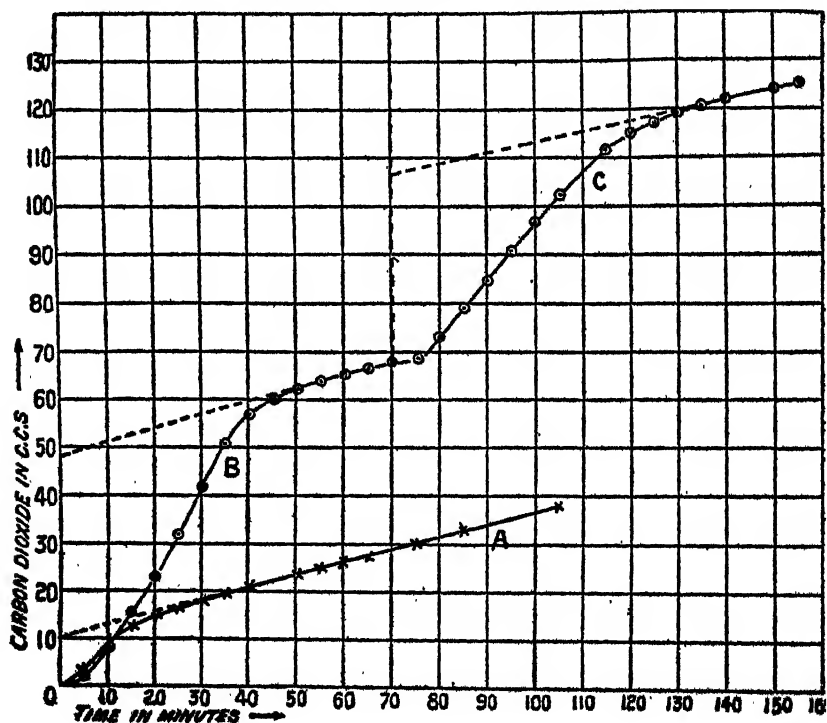
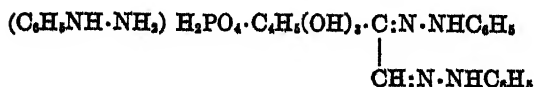
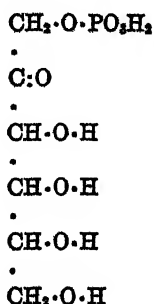


FIG. 1

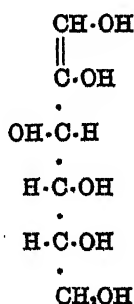
ing the formula: $C_3H_5O_2 \cdot H_2PO_4$ and later showed that this change of inorganic phosphate to organic compounds could be brought about by many of the higher plants. Lebedew, assuming he was dealing with a hexose-mono-phosphoric acid, prepared a phenyl hydrazine derivative of the supposed formula $C_{24}H_{31}N_6O_7P$, but again Harden and Young were able to produce conclusive proof that the substance was the phenyl hydrazine salt of a hexosephosphoric acid osazone of the formula:



That the carbohydrate phosphoric ester is, nevertheless, really a hexosediphosphate:



(where we leave open the question, which of the H-atoms is replaced by the second phosphoric acid radical) was established by Young when he showed that in the reaction with phenylhydrazine there was the separation of one mol of phosphoric acid. Also, the later degradation to the hexose-mono-phosphoric ester, $\text{C}_6\text{H}_{11}\text{O}_6\cdot\text{H}_2\text{PO}_4$ by Neuberg, can be viewed as further making clear the nature of the diphosphates of d-glucose, d-fructose, and d-mannose, which are corresponding "zymophosphates," but we do not know with much precision, as yet, the configuration of the hexose present in the ester. The fact that the γ -fructose present in cane sugar is partially enolized, suggests, by all means, the possibility of the enol formula:



The possibility of a re-formation into fructose as shown by equation (3) forces us to consider, even if we accept the view of Harden and Young, that the hexose-diphosphate is no decomposition product but perhaps an especially reactive compound still retaining six carbon atoms. This conception is supported by the findings of L. Iwanow, v. Euler, and Johansson, who have shown that the formation of hexose-diphosphate can take place independently of fermentation. v. Euler, Kullberg, and Olsén, ascribed this effect to an enzyme which does not act reversibly. This enzyme may be identical with the synthease earlier assumed by Iwanow. As long as we are not authorized to see a conciliation of the contradictions in a manner analogous, perchance, to the findings according to which the fact of fermentation of *composite* sugars by the effect of specific zymases without preliminary hydrolysis is due to a considerably greater number of zymases as hitherto supposed—as long as that condition exists, there remain differences of great magnitude between the two conceptions and the significance of phosphate esters in the course of alcoholic fermentation, in contrast with the change of substances in muscles, is for the present not clear.

The grounds for this are as follows: It is taken as proved that zymophosphates are, in general, unfermentable by living yeast and also that the phosphoric esters can be obtained only by means of yeast juice or dried yeast. If, therefore, the formation of the phosphoric ester be possible without fermentation, it remains questionable whether the two phenomena are to be considered dependent upon each other respectively, whether it would be likely suitable conclusions would be drawn from the constant proportion between the fermented and phosphorated sugar.

If we further consider that an accumulation of hexose-diphosphate takes place only in the presence of an unusually large amount of salts of phosphoric-acid, as well as in the case of one of the normal alcoholic fermentations having an unfavorable hydrogen ion concentration ($\text{pH} = 6.4$),³ then it was interesting to become acquainted with the opinion, that we perhaps have

³ According to Haegglund and Augustson (Biochem. Z., 155, 334, (1925)) the highest fermentative activity of living yeast is attained at $\text{pH} = 4.5$.

to do no more with the usual fermentation process but with an abnormal one which will be discussed later, with one similar to the peculiar general kind of forced glycerol fermentation.

But these considerations of Neuberg, Faerber, Levite and Schwenk repeatedly referred to since 1917 seem again to require revision since the results of recent experiments by Smedley Maclean, and Hoffert. The experiments of these British investigators indicate that the larger hexose phosphate molecule can not permeate the wall of the yeast cell, but that the sugar and phosphate enter the cell separately and then combine. Since yeast contains one of the enzymes which brings about the synthesis of hexosephosphate, and which does not pass through the cell wall, it seems to be easily intelligible that the hexosephosphates may be demonstrated only in cell-free fermentation after the enzyme is present in the fluid.

According to Paine, however, the sodium phosphate partially permeates the cell wall, and therefore in accordance with Emden's results it may also here be true that the permeability of a physiologic membrane varies as the hydrogen-ion concentration varies.

But of greatest importance for the interpretation of the introduction of the reactions in the three carbon chain series (see section 5), are the results of measurements made by v. Euler and Nilsson, who, in logical valuations of the fundamental investigations of Witzemann as well as of Spoehr, and in agreement with Kuhn and Jakob, apparently have established that in the case of non-enzymatic reactions the reactivity of the zymohexose molecule, especially in the case of fructose, is essentially raised.

This fact agrees with the important observation of Warburg and Yabusoe. According to them there is indeed combustion of fructose in the presence of phosphate ions through molecular oxygen as opposed to glucose, which is not affected. It would be attractive, on the other hand, if we could possibly look on one ester of a γ -sugar, the "Transport form" of the dextrose of Hewitt and Pryde (the structure of which is still unknown) as a result of the effect of the Synthease (see section 2), instead of on the fructose-diphosphate. It will be especially necessary, agreeing with Irvine, to suppose that primary steps of isolated sugar

even in enzymatic processes are not chemical individuals, but labile forms.⁴

3. THE CO-ENZYME

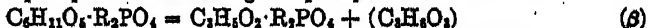
For the initiation of fermentation or phosphorylation, not only are enzymes and mineral phosphates necessary, but also auxiliary systems. Harden and Young (l. c.) in 1906 let yeast juice from top yeast pass through a Martin gelatin filter and thereby obtained an inert residue and an inert filtrate. Buchner and Antoni dialyzed the juice through parchment paper and obtained the same results. If residue and filtrate, each of which can no more decompose sugar, be united, then one obtains a mixture which, in its ability to bring about alcoholic fermentation, is equal to the dialyzed juice. Then besides the zymases there is necessary for the fermentation a substance which is dialyzable and, to a certain extent (according to Tholin until 80°) thermostable. Following the suggestion of Bertrand, Harden and Young called this system a co-enzyme. The inactive residue from the dialysis can be made active again also by the addition of boiled juice or by the addition of an inactive yeast extract termed also apozy-

⁴ Note added at correction:

Raymond (Proc. Nat. Acad. Sci., 11, 622, (1925)) has recently apparently also accepted this viewpoint, assuming that the hexose-mono-phosphoric acid of Robison (Biochem. Journ., 16, 810, (1922)) (which occurs together with hexose-diphosphate but is not identical with the decomposition product of Neuberg) is the intermediate conversion to the ester of hexose-di-phosphoric acid. This takes place through the hexose-mono-phosphoric acid:



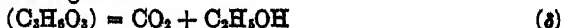
which, splits into a phosphorus-containing and phosphorus-free triose:



and by the condensation on the one hand to hexose-di-phosphoric acid,



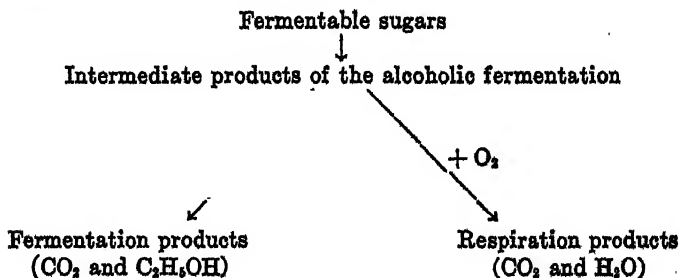
on the other hand by the breaking down of



to alcohol and carbonic acid. However, it is amazing that this particular reactive form is ascribed to the hypothetical triose although the process in the 3-carbon series seems explained and still more because the apparent and experimentally established interpretation of the transition to the 3-carbon series is lacking.

mase. The complex zymases capable of bringing about fermentation are composed, then, of the sum of the coferments plus apozymase, hence the former is one part of an activated system of ferments.

Of fundamental significance is Meyerhof's observation that the co-enzyme of alcoholic fermentations occurs in the muscles and organs of animals as well as in milk. He also showed that aqueous extracts of animal muscles and germinating plants strongly furthered alcoholic fermentation. This seems to be entirely in accord with the earlier observation of Kostytschew, Hübbenet, and Scheloumow that yeast extract increases the normal respiration of plants. Meyerhof, on this basis, suggested that the same co-ferments are necessary for the normal respiration of animal tissue as for yeast fermentation, i.e., it was supposed that hexosephosphate was just as indispensable for the fermentative oxygen respiration of animals as for the fermentative alcoholic fermentation. Although Scandinavian investigators have concentrated the coferment by precipitation on tannin, phosphotungstic, and silicotungstic acids, the character of the systems remains poorly known. Among all the different interpretations there is to be noted the result of Meyerhof, who suggested the probability that between fermentation and respiration there exists a connection. This view can be shown schematically, according to Kostytschew (l.c.) as follows:



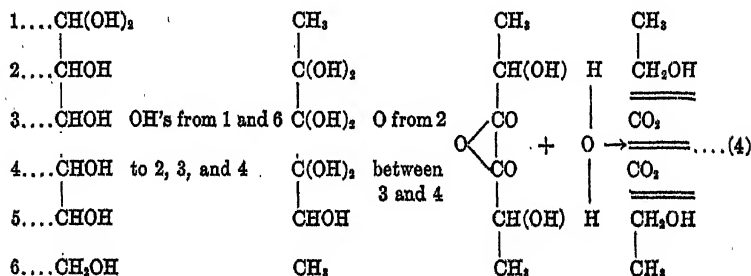
and it is finally based on the classical hypothesis of Palladin according to which the oxidation and reduction ferments of vegetable tissue transform the primary products of sugar split-

ting, which have been previously formed by the enzymes of fermentation, to the end-products.

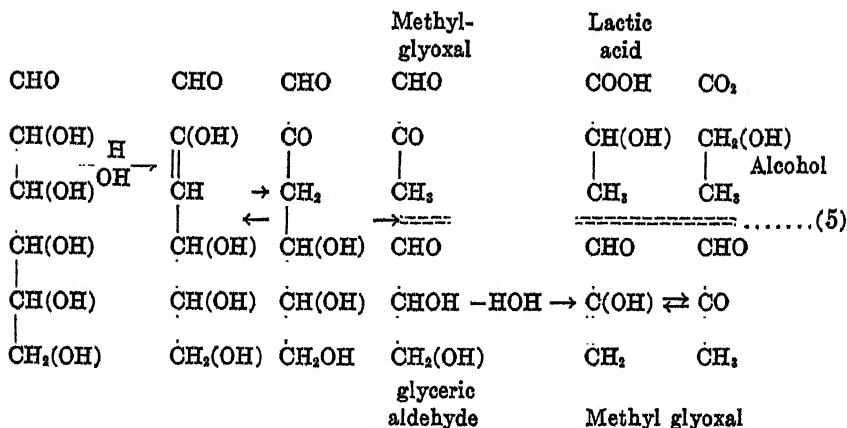
In view of the present confusion in the field of the "kinases" we are unable to state definitely whether the systems in question represent a real auxiliary catalyzer, although v. Szent-Györgyi, for example, has stated that he was able to replace co-ferments by definite substances (p-phenylenediamine). (Compare also page 57.)

4. THE TRANSFORMATION *into* THE THREE CARBON CHAIN SERIES

In 1870 v. Baeyer expressed the assumption that in the course of sugar decomposition the splitting out of water in one place in the sugar molecule and the addition of the same in another plays an important rôle. If one further assumes an oxygen migration from the end to the middle of the molecule, one can derive a schematic way of showing the formation of lactic acid as well as alcohol and CO_2 :

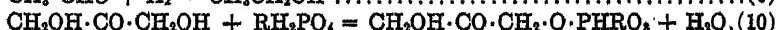
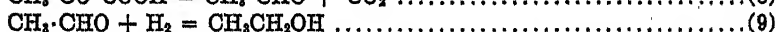
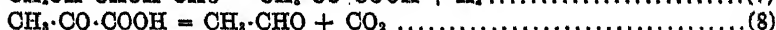
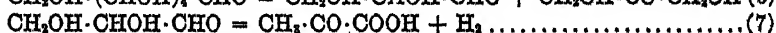


From considerations based on investigations carried out by Wohl and Österlin, Baeyer's assumed displacement of oxygen from the end to the middle of the chain could no longer be considered the thing that takes place in fermentative reactions. The central idea of the scheme proposed by Wohl in 1904—in its characteristic features it is still considered valid—rested on the fact that in hydroxy compounds there can readily be a splitting out of water.



The six carbon compound which is believed to be ready for decomposition in this scheme is the methyl glyoxal-glyceric aldehyde aldol, which would form, through an easy hydrolytic splitting, two fragments of three carbons each, viz., methyl glyoxal and glyceric aldehyde. As an illustration of the relationship it may be recalled that Pinkus observed the formation of methyl glyoxal when glucose was warmed with caustic potash, and Wohl (l. c.) obtained it from glyceric aldehyde itself.

v. Lebedew and Griaznoff made another deduction in which they take the stand that the hexose molecule decomposes into glyceric aldehyde and dihydroxyacetone. In the further course of the change, it is considered that the glyceric aldehyde forms the easily fermented pyruvic acid while the dihydroxy acetone forms a hexose diphosphate:

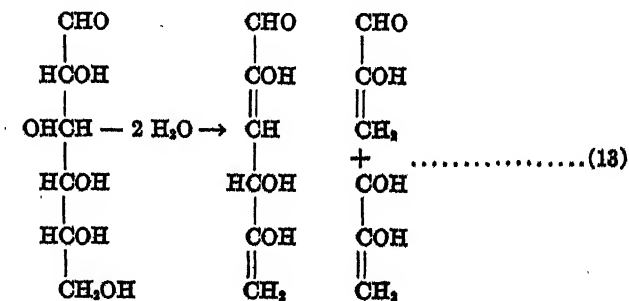


For the time, however, a real objection can be raised as to the correctness of both conceptions. One has a right to assume that if glyceric aldehyde, dihydroxyacetone, or methyl glyoxal are

really the intermediate products of alcoholic fermentation, at least one of these substances must be fermentable. In spite of the work of such well known investigators as Buchner, Emmerling, Färber, H. O. L. Fischer, Harden, Levite, Neuberg, Schwenk, Sclator, Young and others, the uncertainty concerning this question is not removed and lacking clear, valid proof of their fermentability, we are forced to draw also on other compounds of the three carbon series to explain the intermediate steps in the fermentative decomposition of sugar.

Supported by the discovery of Fernbach which was later confirmed by Aubel, viz., that methyl glyoxal could be demonstrated in the carbohydrate splitting by bacteria, Neuberg and Kerb have recommended that the hypothetical formation of methylglyoxal-glyceric aldehyde aldol be replaced in the Wohl scheme by that of methylglyoxal-aldol. The split products of the former compound have been shown, as yet, through biological means, only partly to fit into the deductions of the fermentation reactions.

For avoidance of the glyceric aldehyde, this change makes possible, in the transformation into the three carbon chain series, the assumption of the formation of two mols of methyl glyoxal from each mol of glucose:



If we once more consider that the acid corresponding to methyl glyoxal is pyruvic acid,



and that the relation of this acid to glucose, not only chemically

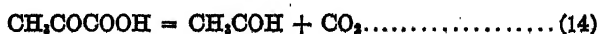
but also in its ease of fermentation, is established, then we are in a position, when we consider the combination of this assumption with the important observations by Battelli and Stern, Embden and Baldes, Trillat and Sauton, Wieland and Kostytschew (according to whom aldehydes can undergo fermentatively the Cannizzaro reaction), to find a way for explaining the formation of the end products of alcoholic fermentation.

5. THE ORIGIN OF THE END PRODUCTS

At the beginning of this century there was a hypothesis expressed and an investigation reported which have influenced up to the present and in undiminished strength the development of the chemistry of fermentation. The hypothesis and research developed entirely independently of each other. In the course of his fundamental researches concerning metabolism, Magnus-Levy expressed on March 14, 1902, the view that acetaldehyde is a split product of carbohydrate, and on November 1, 1920, O. Neubauer, in connection with the comprehensive investigations by Neubauer and Fromherz concerning the decomposition of amino acids by yeast fermentation, made in the form of an unpretentious note the following communication:

Weiter ist zu schliessen, dass die hier als Zwischenprodukt auftretende Brenztraubensaure durch gaerende Hefe unter Reduktion zu Kohlensaure und Alkohol zersetzt wird, d. h. mit anderen Worten, dass sie leicht vergaerbar sein muss. Eigens angestellte Versuche, die noch nicht voellig abgeschlossen sind, haben die Richtigkeit dieses Schlusses bestaetigt. Damit ist nun ohne weiteres der Gedanke gegeben, die Brenztraubensaure koennte ein Zwischenprodukt bei der alkoholischen Gaerung des Zuckers sein.

Later Neuberg and Karczag were able to prove the principal part of this communication by showing that the fermentation of pyruvic acid proceeded according to the simple equation:



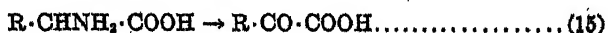
This reaction is brought about by the enzyme carboxylase which occurs in all zymases and takes place with no giving up of free

energy. It represents a typical case of an enzymatic breaking down of a carbon chain.

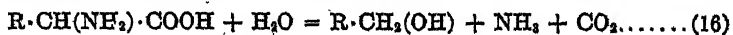
The zymases necessary for the carrying out of this splitting are no simple ferments but enzyme complexes in which the pyruvic acid splitting ferment, the carboxylase, occurs. The pyruvic acid, however, is, on the contrary, potentially represented in glucose or other fermentable carbohydrates. Through the important fact that carboxylase can break a carbon chain, this ferment assumes a special place among the known enzymes. In the fermentation of pyruvic acid, i.e., in the case of a non-sugar undergoing by means of yeast a characteristic change, we see the first case of a sugar-free yeast fermentation.

The essence of this process is that the carboxylase splits out CO_2 from α -keto acids and α -keto dicarboxylic acids, leaving behind as a residue the aldehyde of the next lower series. This aldehyde is left in an unusually reactive form.

But through the circumstance that pyruvic acid not only stands in many close relationships to glucose (see page 54 and below), but also, for example, is the α -keto acid corresponding to alanine (as ketoglycerolacid to serine), there is some indication that carboxylase may have a predominant place among the proteolytic enzymes since we know from experiments of Neubauer and Fromherz mentioned above, that the amino-acids go over, in general, intermediately into the corresponding ketonic acids:

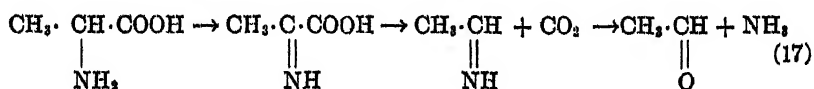


by splitting of the amino groups. The ketonic acids in turn by alcoholic fermentation liberate similar alcohols which according to Ehrlich are produced by the fermentation of amino-acids only by living yeast and in the presence of a large amount of sugar:



But it remains undecided, whether in this process the cleavage of the NH_2 -group takes place first and then the α -Keto acid is formed, or whether in the sense of the models of Wieland and Bergel the amino group is converted to imine and later, having

lost carbondioxyde, the hydrolytic separation of the <NH-group



follows.

We must further consider that the transformation of pyruvic acid to lactic acid produces an increased hydrogen ion concentration (since the dissociation of pyruvic acid is 3.6×10^{-3} , that of lactic acids 1.38×10^{-4}). The fermentation of salts of pyruvic acid, on the other hand, brings about a change of the hydroxyl ion concentration, so, according to Neuberg, there can be no exclusion for the assumption that the carboxylase, through the separation of CO_2 , executes the function of regulating the reaction of the fermentation medium.

The connecting rôle of α -keto acids, which represent common ground between proteins and sugar, manifests itself also in the phenomenon that these acids serve as stimulators in alcoholic fermentation. This is believed to have been proved also by experiments which aroused the impression that the co-enzyme of yeast, in the presence of potassium phosphate, can be replaced by a mixture of different α -keto acids such as occur in yeast protein. Meyerhof was not able to confirm this statement using pyruvic acid. (Compare also v. Szent-Gyögyi, l. c.)

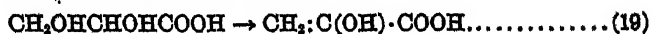
The fermentability of pyruvic acid conditioned by its relative nonpoisonous nature, or the properties of the above mentioned decomposition products, constitutes the unrestrained confirmation of Neubauer's assumption and experiments that this acid is an intermediate product in alcoholic fermentation, or a split product of the sugar molecule, for, in contrast to its ability to withstand the action of a temperature up to 165° and of concentrated sulfuric acid up to 150° , in the presence of various yeasts, it is, in a few minutes, split (70 to 80 per cent) into CO_2 and acetaldehyde.

But apart from the biological and earlier (page 53) discussed chemical connections, there is also a hint of the genetic connection

to the glucose in that it may be formed endothermically by the oxidation of lactic acid,



or by the dehydration of glyceric acid

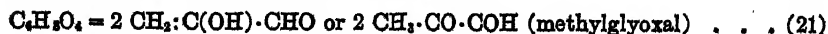


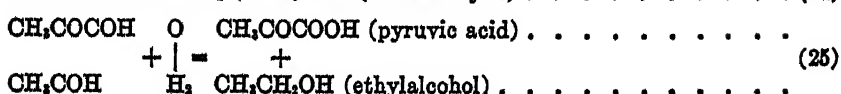
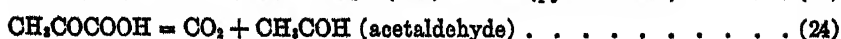
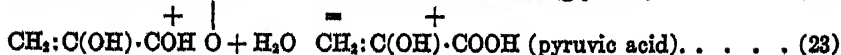
In the above given scheme of decomposition we became acquainted with the compounds in which carbonyl and ethylidene radicals are represented so that their origin is withdrawn from "paper chemistry" considerations, and is placed within the range of experimental proof.

The connection between the assumption of Magnus-Levy and the discovery by Neubauer, or Neuberg and Karczag, is therefore clear at first sight, and in the case of the fermentation process it can be established that the proof of the origin of CO_2 is given. Biologically speaking, it is the same whether acetaldehyde and CO_2 occur as such or are held fast in the form of their combination, i.e., pyruvic acid. (Compare section 6.)

Not as simple is the explanation of the formation of alcohol. It has been shown by Battelli and Stern (l. c.) and by Parnas that there occurs in animal tissues a ferment which acts upon two mols of an aldehyde with the taking up of water to give a Cannizzaro reaction, i.e., the aldehyde is rearranged to equal molecular amounts of alcohol and acid. An analogous observation was later made, also, by Kostytschew (l. c.) in the case of the action of yeast on acetaldehyde. But a Cannizzaro reaction on the acetaldehyde formed in the decomposition of pyruvic acid gives ethyl alcohol itself.

By means of a suitable arrangement of the above mentioned discoveries, and taking the sugar from the decomposition of hexose diphosphate to be in an especially reactive form (see page 49) the earlier discussed scheme of Wohl (see page 53) could be completed in the following way:

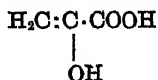




Omitting processes experimentally not clear at present, processes which are concerned in the formation of the three carbon chain series, methylglyoxal is considered the first product having three carbon atoms in the molecule. It may be formed after the splitting out of two molecules of water from the hexose molecule, which requires perhaps the intermediary existence of methyl glyoxal aldol. All further processes then are brought about through repeated Cannizzaro rearrangements on methyl glyoxal. In case of the pyruvic acid first formed, the addition of water gives glycerin. If we assume as isochronous the formation of acetaldehyde and CO_2 by the action of carboxylase on the pyruvic acid, then a Cannizzaro reaction must take place between two *different* aldehydes. The result of this phenomenon is the formation of ethyl alcohol on the one hand and the re-formation of pyruvic acid on the other. From the latter the carboxylase always produces new CO_2 and acetaldehyde. We see, therefore, an uninterrupted formation and decomposition of pyruvic acid, which, just as methyl glyoxal, can not then be accumulated. After this restless conversion naturally some acetaldehyde would be left over. This assumption is in harmony with the fact that in fermentations there are always detectable traces of acetaldehyde equivalent to some 0.15 per cent of the raw materials used.

One has the impression that the above mentioned facts may also be supported by the important findings of Henri and Fromageot. These investigators have shown, by measuring the absorption spectra of pyruvic acid that the quantitative relations of the keto and enol forms of this acid, under conditions of concentration that approach closely to those of biological processes are strongly dependent upon the hydrogen ion concentration.

The much more reactive enol-form



could thus also be responsible for the fermentability of the acid in normal fermentation media.

But, when one considers the above statements in connection with the repeated assertion that the velocity of fermentation of pyruvic acid, at ordinary temperature can several times exceed that of glucose, which has been deduced from experiments carried out mainly in the presence of salts of sulphurous acid, in which the hydrogen ion concentration was not measured, one will not be able to rid himself of a certain feeling of uncertainty as to the actual rôle of pyruvic acid in these processes.

Before we enter into the discussion of the evidences upon which the experimental basis of this scheme rests (see section 6), let us first inspect the instances in which critical consideration could see further difficulties:

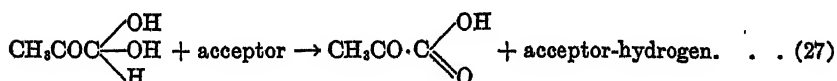
It is quite true that the fermentability of methylglyoxal is not as yet demonstrated, but nevertheless this circumstance can not speak conclusively against the assumption that the compound can be an intermediate in alcoholic fermentation, since Neuberg and Hirsch suppose that it shows a surprising number of different isomeric forms, which might have a different behavior when acted upon by yeast. It should, moreover, be mentioned that Dakin and Dudley, even as Neuberg, state that they have found in yeast, muscle and other animal organs, an enzyme which brings about an internal Cannizzaro reaction:



thereby forming lactic acid, which appears, as is known, in the case of fermentation of sugar by yeast juice. Therefore, there are chemical (see page 54) and biological grounds which do not exclude the right for assuming the intermediate appearance of methylglyoxal.

In some communications worthy of note but not yet adequately backed by experiment, Kluyver and Donker have recently turned

against the idea of a mixed Cannizzaro reaction. The essential thing in their theory, in which they attempted to apply Wieland's theory of dehydrogenation in processes of aerobic respiration to anaerobic dissimilation is that all parts in the process of sugar decomposition are none other than coupled dehydrogenation-hydrogenation reactions. Correspondingly, alcohol would be formed from a dehydrogenation of methylglyoxal hydrate through passage of the hydrogen to the acetaldehyde.⁵



They state: "On the basis of these facts it is evident that there exists no reason whatever to have recourse in certain cases to the narrower explanation offered by a Cannizzaro transformation."

We shall see later that these explanations are automatically weakened by experiment (section 6, paragraph 2).

6. EVIDENCES

Dumas early showed that alcoholic fermentation can proceed in the presence of various alkali salts, and in 1874 he hinted the correctness of the possibility of influencing the alcoholic sugar decomposition through the presence of alkali sulfitcs. Later Mueller-Thurgau and Osterwalder observed that in the case of fruit juices or fermenting sugar solutions, added sulfurous acid instantly combined with the—as they correctly assumed—acetaldehyde present. It was obvious that this compound might be the acetaldehyde-sulfurous acid of Ripper (1892), of which the sodium salt, known since the time of Bunte (1873), is so important for our following discussion.

In the course of extensive investigations concerning the hydrolytic splitting of bound sulfurous acid, Kerp and Laudon have

⁵ Compare also A. Lebedew, Bull. soc. chim. France, [4] 11/12, 1040, (1912).

established that the dissociation constant of acetaldehyde sodium bisulfite (2.84×10^{-6}) to the corresponding glucose compound (311×10^{-8}) was an average of about 1:90,000.

Proceeding from the knowledge of the above mentioned facts and from the desire to increase as much as possible the yield of glycerol in alcoholic fermentation (see page 59), Connstein and Luedecke began pertinent experiments in 1914. The ordinary fermentation of sugar takes place always either in neutral or slightly acid solution. Should, however, the possibility exist of removing the acetaldehyde formed (equation 24) as Bunte's compound, it was to be expected that in the case of an appropriate method of fermentation or experimental conditions, not inconsiderable amounts of glycerol could be obtained.

The first investigations were made with various alkaline substances as disodium phosphate, sodium acetate, sodium bicarbonate, ammonium carbonate, etc. However, the alkaline mash brought to light a disagreeable characteristic in a short time, viz., these alkaline mash formed an excellent nutrient medium for all possible acid forming bacteria, especially lactic acid bacteria. These acid forming bacteria consumed not only the greater part of the sugar, but also made the glycerin so impure that it was very difficult to purify. Then were begun the investigations of the manufacture on a large scale with disodium sulfite, which salt the yeast tolerated in increased quantities, and, when added in larger amounts to goods to be fermented, exerted a pronounced antiseptic action.

A. With just a hint as to the complete explanation found elsewhere, the mention of technical investigations in this case has significance. Here is displayed a not frequent case where the practical workers have very fruitfully and stimulatingly influenced theoretical research in this sphere. Chemists in the United States (Eoff, Lindner and Bayer), Germany, England (Cocking and Lilly), the former Austria-Hungary, Switzerland (Schweizer), and Japan (Tomoda), vied with each other in the endeavor to solve the problem, and it remains to the merit of Faerber, Hirsch, Neuberg, Reinfurth and Ursum on the one

side and Pollak, Reik, and Zerner, on the other, to have produced a correct interpretation and a part of the proof for the scheme given on page 58.

Starting out from the long opposed observation of Fernbach and Schoen, that pyruvic acid occurs among the products of alcoholic sugar decomposition, it could be proved that if one carries out the fermentation in the presence of the weakly alkaline alkali sulfites, the theoretically possible amounts of acetaldehyde and glycerin are obtained. The already mentioned aldehyde-bisulfite addition compound, $\text{CH}_3\cdot\text{CHOH}\cdot\text{O}\cdot\text{SO}_2\text{Na}$, concentrates itself in the mashes and can be separated. The corresponding hexose compound in the presence of water is practically completely dissociated. Compared with ethyl alcohol, acetaldehyde is considered as an oxidation product, and hence the presence of a reducing compound is to be expected. This assumption really proved correct, since the hydrogen atoms, which can not act upon the acetaldehyde because of its formation of a compound with Na_2SO_3 , make possible the formation of glycerol by acting upon another half molecule of sugar:



Detached from the binding to the alkali salt, the equation takes the form:



and according to the curve in figure 2 (Biochem. Z. 98, 153) the ratio between glycerin and acetaldehyde at any given time a constant.

It is quite worthy of note that the blocking of the acetaldehyde can be brought about not only in a chemical way but also, as Abderhalden, Glaubach and Stix showed, through adsorption. They have proven that the above reactions can be made to proceed in an approximate manner in the presence of animal charcoal.

If the fermentation be carried out in the presence of simple alkali salts which, in contrast to the bisulfites, have no specifi-

cation on the acetaldehyde formed, only traces of the latter can be found. It undergoes a Cannizzaro transformation and can be found again also in molecular proportion to glycerol, but as acetic acid and ethyl alcohol. The proportions can be expressed in the following equation:

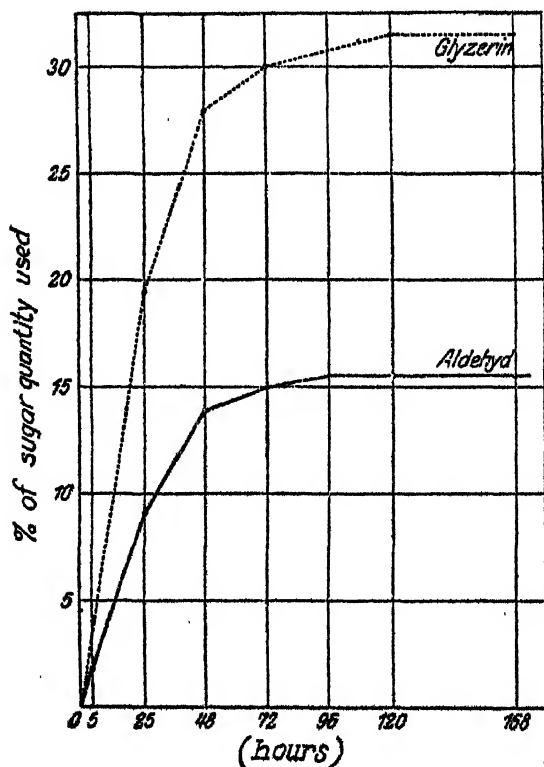
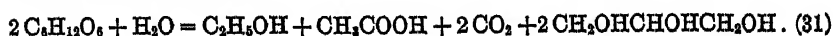


FIG. 2

and show that for every mol of acetic acid there are always two mols of glycerol.

On the basis of the above facts, v. Grab working with C. Neuberg, could confirm the statements of Fernbach and Schoen by carrying out a Doebner synthesis of α -methyl- β -naphtho-cinchonic

acid in a cell-free fermentation of sugar. This was done by the reaction of β -naphthylamine with pyruvic acid:



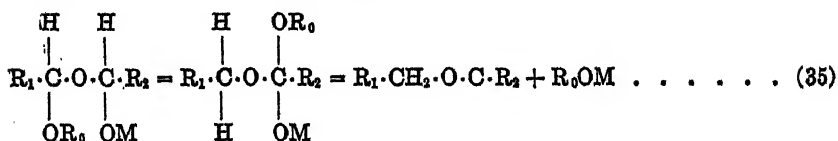
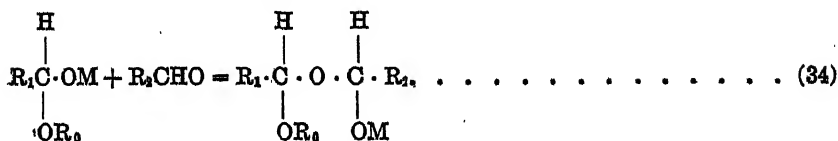
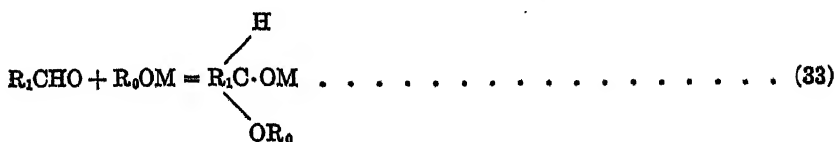
On the other hand Aubel and Salabartan showed that pyruvic acid was an intermediate product in the Coli fermentation of glucose (see below). When we take all these things into consideration this part of the demonstration may be considered closed although Lebedew expressed doubt about the data produced by v. Grab.

B. After the above discussion (not mentioning equations 20 and 21 on page 58) there remains in doubt only the correctness of the assumption of a Cannizzaro reaction between two *different* aldehydes. Several investigators, indeed, have pointed out the necessity of proof to strengthen the assumptions involved.

During the last years extensive investigations for showing this reaction were taken up by Endoh, Nakai, Nord, and others, and in 1921 v. Grab, stating (l. c., page 71): “. . . durch den von Nord erbrachten Nachweis, dass in der Tat eine gemischte Dismutation zwischen ungleichen Aldehyden der aliphatischen Reihe moeglich ist,” was able to rely upon the feasibility, as founded on certain principles, of the assumption of a mixed Cannizzaro reaction; afterward the preliminary work of the Lieben school did not clear up the question.

Meanwhile these catalytically influenced model investigations have been completed, and we now know the following examples: acetaldehyde plus isovaleric aldehyde, isovaleric aldehyde plus benzaldehyde, acetaldehyde plus benzaldehyde, acetaldehyde plus furfural, furfural plus isobutyric aldehyde, acetaldehyde plus chloral, acetaldehyde plus bromal, acetaldehyde plus cinnamic aldehyde. Indeed, the reaction can take place with the most different kinds of aldehydes and we were authorized already by the indications of Verley to assume that this reaction might also occur with mixtures of aldehydes *and* ketones.

Simultaneously, the investigations have brought two other conclusions to maturity. (a) Through the formulation:



which is based upon the work of Wieland or Lachmann, it is probable that an *isochronic* rearrangement of dialkyl ethers precedes the formation of mixed esters. (b) By the application of the method to single aldehydes, several alcohols of importance in physiological investigations could readily be made accessible.

7. REDUCTIONS AND SYNTHESSES IN THE COURSE OF FERMENTATIONS

A. Of the biochemical processes found in nature, the reduction processes are of greatest interest because they are the most difficult to interpret. We see, that beside the deoxidation of CO_2 in assimilation processes, plants bring about the greatest hydrogenation performance in the synthesis of protein from sugar and ammonia, as well as in many other processes. One has an object worthy of study in the reductions done by yeast cells. Since these reductions, because of lack of disposable hydrogen, can be brought about only in roundabout ways—one often thinks of so-called coupled or induced reactions—the question is often asked, from where does the energy for the reduction processes come? A whole series of biological experiences assign a connection between reductions and the oxidative decomposition of car-

bohydrates, and especially the processes of fermentation which have been discussed appear as an alternate oxidation and reduction of the sugar molecule.

In continuation of the basic observation of Lintner and v. Liebig concerning the reduction of furfural by yeasts in alcoholic fermentation, the reductive action of yeast on other aldehydes was made the object of further work and it could be shown that this characteristic is so marked that the yeast could even act upon substances foreign to their organism. Experiments of "phytochemical" reductions made with various nitro compound showed the formation of the amino compounds by way of the corresponding intermediate compounds and in the case of o-nitrobenzaldehyde, for example, it could be shown that the yeast could also act selectively. This is in harmony with the experiences of Nord on the catalytic hydrogenation in steps.

Several years ago it was stated in a lecture that the reducing power of yeast could also act on ketones. The reaction takes place as with methylheptenone in both the aliphatic and aromatic series, and as it is an assymetric one, it furnishes a method of obtaining optically active secondary alcohols which would otherwise be difficult to obtain. Diketones undergo the same change and give glycols. Examples of this class which have been studied are diacetyl and benzil. It is definitely proven that these reductions are not purely chemical in nature, but are catalyzed by ferments, by the fact that if one uses a racemic aldehyde, e.g., racemic valeric aldehyde, one gets optically active amylalcohol, or, if one begins with diacetyl, one gets optically active butylene glycol.

The alcohols in nature result from the corresponding carbonyl compounds, i.e., from aldehydes and ketones. As for the thioalcohols, the mercaptans, the possibility of their origin from the thioaldehydes could also be substantiated. For this purpose one uses in the phytochemical reduction of the thioaldehydes their ammonia derivatives, the thialdines. In this case it must be evident that the origin of these compounds in nature appears entirely possible, as ammonium salts are available everywhere.

In the case of ethyl mercaptan, however, it could be shown that the formation of the mercaptan is purely an enzymatic process, as it can be brought about in cell-free fermentations by use of yeast juice.

In the course of these investigations there is acquiescence to the idea that the reduction of various compounds has a relation to the simultaneous course of the processes of alcoholic sugar decomposition, pointing to the possibility that in ultimate analysis the "hydrogen" used in reductions is fermentation "hydrogen," which, naturally, is not evolved in the free state and therefore requires an acceptor.⁶

Since the course of sugar decomposition seems to involve pyruvic acid and acetaldehyde as intermediate steps, and since the former is an oxidation product of half a sugar molecule, it is necessary, if there is to be any change in this oxidation product, for an equivalent reduction process to occur simultaneously.

Normally the acceptor necessary is formed through the further splitting of the pyruvic acid, occurring in the form of acetaldehyde which takes up the "hydrogen" and passes over to alcohol. If this representation is correct, other hydrogen acceptors, i.e., phytochemically reducible substances, must work in a manner analogous to acetaldehyde.

But the fact of the reduction of many compounds in itself says nothing concerning the course of the process. The yield of the reduction product only teaches that no process like a Cannizzaro rearrangement can come into play, not mentioning that simple ketones and nitro compounds are not in general capable of a transmutation. We are inclined, then, to draw the fact into consideration that it has been possible to demonstrate acetaldehyde in such biochemical fermentations. In singular cases the acetaldehyde has been found in amounts almost equivalent to the reduction product, so, one receives the conception that here indeed the added reducible compound has become the acceptor

⁶ Compare in the case of sulfur the interesting statement of A. Hottinger (Schweiz. med. Wchnschr., 53, 430, (1923)).

for the fermentation hydrogen instead of the acetaldehyde and, in agreement with that, has pushed aside out of the reaction an equivalent amount of acetaldehyde.

It is interesting to note in this connection the fine demonstration of the origin of acetylmethyl carbinol and 2, 3-butylene glycol, corresponding to the earlier results of Harden, Walpole, and Norris, by Kluyver and Donker, in the fermentation of glucose in the presence of methylene-blue or sulfur. The products of decomposition occurring in the fermentation of fructose have, in this sense, also the effect of a hydrogen acceptor.

If with these authors, we include oxygen in this sphere, the aforementioned oxidation phenomena would in addition to this take on a very original and attractive interpretation (see page 43).

But we must remember also that yeast co-ferment can pretendedly be replaced by a mixture of various α -keto acids (see page 57) (but apparently not by insulin). According to Kendall, thyroxin acts as a catalyst which exercises its action by being alternately oxidized and reduced; that is, it acts simultaneously as donator and acceptor.⁷ Should it be found that thyroxin and the bios isolated from autolized yeast by Eddy, Kerr, and Williams, are joined in relationship on the basis suggested by Kendall that the active group in both these (and other)

compounds is $\overset{\text{C}}{\text{C}}-\overset{\text{N}}{\text{N}}$ - then perhaps further investigations may put us in a position to seek in this direction for an explanation of the reductive processes in yeasts.

B. Reference has already been made (see page 56) to the fact that zymases are not uniform, but constitutes enzyme complexes in which various tasks fall to the different parts. It is interesting to note, as ways of checking up this opinion increase in number, the things that can be drawn up as confirmation for this assumption. In the fundamental investigations of Lintner and v. Liebig the observation was made that a considerable part of the furfural added to a fermenting sugar solution was not reduced

⁷ Compare also F. Knoop and H. Oesterlin, *Z. physiol. Chem.*, **148**, 301, 302, (1925).

in the way described above, but apparently underwent a condensation by the acetaldehyde which appeared, as an intermediary. In continued investigations they showed that the by-product was in reality the result of a direct carbon chain linkage, C-C, and, since the compound called by them furyltrimethylene-glycol, $C_4H_5O \cdot CHOH \cdot CH_2CH_2OH$, was optically active, there could be no doubt that it was the result of fermentative action. In contrast to the ordinary process of biochemical breaking down of molecules, which we regard as the function of fermentation, here we see established the first case of a carbon chain synthesis or the carboligation action of a ferment, hitherto recognized only in its performances.

If one replaces the furfural with benzaldehyde, optically active phenyl acetyl carbinol, (α -Phenyl-pryuvic alcohol) $C_6H_5C^*HOH \cdot COCH_3$, is formed, and, in the fermentation of pyruvic acid alone there occurs according to Hirsch, in addition to acetaldehyde, methylacetyl carbinol (Acetoin)



All these reactions indicate that they can take place only with nascent acetaldehyde. Neuberg and v. May could also show this by fixing the acetaldehyde by sulfite whereby they obtained a total suppression of the carboligation action. But the process of carboligation itself is not connected with the aldol reaction and it is indicated in comparison to the Benzoin condensation as "Acyloinreaction."

There occurs therefore the conversion of an oxidative splitting process under raising of the potential into the direction of a fermentative resynthesis of carbohydrate, for in all these cases of transformations there occurs sugar or pyruvic acid which is being decomposed to give the necessary acetaldehyde for the carboligation effect.

But also, the view of Haehn that acetaldehyde forms also a fundamental ingredient of fats, is confirmed through the origin of higher fatty acids, when the organism, *endomyces vernalis* is used. In opposition to Smedley Maclean and Hoffert, Haehn and Kintoff have proved that the building up of the fatty acid

* C indicates an assymetric C-Atom.

molecule is accomplished in two steps, the first by the decomposition of glucose to acetaldehyde through the action of the zymases, the second by the action of the synthesizing enzymes on this aldehyde. It is impossible to state whether the pyruvic acid or lactic acid precedes acetaldehyde. The above mentioned fungus has shown itself able to assimilate both acids as well as the aldehyde.

8. BACTERIAL FERMENTATION

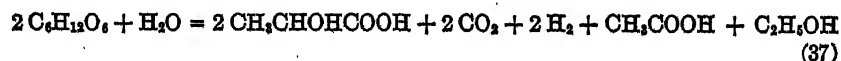
Not only in the case of alcoholic fermentation does acetaldehyde, as shown above, play an important rôle (by being one step in alcohol production) but also in cases of important bacterial fermentations in nature, the same is equally true. In order to show this, one permits the bacterial process concerned to proceed in the presence of a substance which will fix the expected aldehyde. For this purpose it has been shown that, in the case of bacterial fermentations, the neutral sulfitcs, as Na_2SO_3 and CaSO_3 , can be used. They, in contrast with most of the bisulfitcs required for the fixation of aldehydes, cause little injury to the enzymatic and life processes, or in any case, do not stop them. The whole difficulty of this problem consists in this, that the expected intermediate products which are formed during the disintegration of sugar to its split products, acetaldehyde and alcohol, all show labile characteristics, and, collectively, must contain the carbonyl ($-\text{CO}-$) group. Because of that, the choice of appropriate means of intercepting the reaction is extraordinarily limited. The compounds used must combine already with the sugar to form a substance not readily acted upon biologically or else, under the conditions of the experiment, give no condensation product at all. This obstacle blocked all of the earlier steps attempted in this direction. The way out of this difficulty consists in selecting a compound which will intercept the reaction, one which has a fine gradation in its affinity for sugar and its different transformation products so that the least affinity is manifested for the initial material and the maximum for one of the later products of this change. (Compare Kerp and Laudon, page 61.) Such substances, as mentioned above, are the normal

sulfites. Sulfites give with sugar an addition product in which the components are exceedingly loosely bound, and which, in water solution are highly dissociated. On the other hand, the stability of the sulfite complex increases, down to acetaldehyde. As normal sulfites (M_2SO_3) are not poisonous for microorganisms, it is possible to carry out fermentation in their presence. This is different from free sulfurous acid (H_2SO_3) and bisulfites ($MHSO_3$) which, because of their disinfecting qualities, are used in the different industries based on fermentation.

By this method Neuberg and Nord were able to show that acetaldehyde occurred as an intermediate product in the fermentation of sugar, mannite, and glycerol, by means of the widespread kinds of coli and various pathogenic microorganisms.

The exchange of carbohydrates through means of these agents is, in nature, very great in scope. Their action extends not only to the true sugars but also to related substances as mannite and glycerol. The yeasts are relatively strongly constituted organisms which will stand the addition of a substance which takes up some substance formed in the course of the reaction. In the case of bacteria it is necessary to make a modification, which consists of using an alkali-earth sulfite (as $CaSO_3$) which gives a neutral reaction instead of the alkali metal sulfites which have a basic reaction. The former not only have the advantage of being neutral in reaction, but also are relatively insoluble in water. Because of that, one can exclude, in the case of sensitive microorganisms, detrimental osmotic processes. On the other hand, one obtains only a diminished sulfite-ion concentration which might be adjusted to a certain degree, through mechanical means. The presence of a sulfite is the only essential.

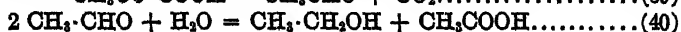
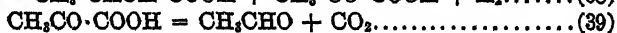
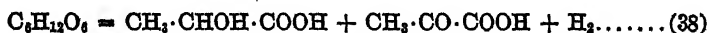
The aldehyde clearly is produced at the expense of the reaction which normally leads to ethyl alcohol or acetic acid production. Harden's investigation of the coli fermentation gave as the normal reaction:



In the presence of sulfite, aldehyde in the appreciable amount of

40 to 45 per cent of the quantity of alcohol concerned, was obtained.

But the aforementioned authors believe that the acetic acid and the alcohol are derived by a Cannizzaro reaction from acetaldehyde. If this is true the equation given above for the breaking up of the glucose by colon bacilli may be interpreted to mean that pyruvic acid and acetaldehyde come next. This gives the following equations:



The similarity which has often been noted between fermentation of yeast and colon bacilli would be based on the fact that in the latter, also, that part of the sugar which does not change into lactic acid is decomposed by way of pyruvic acid and acetaldehyde. The reduction of acetaldehyde in the last phase does not take place in fermentation by colon bacilli because the hydrogenizing hydrogen is developed freely in the molecular state while at the same time the acetaldehyde primarily produced becomes a simple transformation into alcohol and acetic acid.

The recent results of de Graaff and LeFevre seem to coincide with this viewpoint. According to them the methylglyoxal is not decomposed by the bacteria of the colon-typhoid bacillus group by way of acetaldehyde. On the other hand, Neuberg and Gorr noted the effect of lactic acid bacteria (*B. coli*) on methylglyoxal and found that this unstable compound was stabilized to lactic acid, just as Aubel and Salabartan (l. c.) had noted in their observations.

The same information obtained with the coli bacteria was also obtained for the metabolic processes of two pathogenic microorganisms, viz., the dysentery bacillus and the gas gangrene bacteria. The known kinds of dysentery bacilli, the Flexner, Y, and Shiga-Kruse, all give acetaldehyde in cultivation in solutions of maltose, glycerol, and mannite. The causal organism of gas gangrene, which in the late world-war was so feared in wound infection, is likewise a powerful carbohydrate consumer. This

anaërobic organism related to the butyric acid bacteria grows in sugar solutions with the production of acetaldehyde. Since acetaldehyde is a violent poison for higher organisms, its occurrence amongst the products of metabolism of pathogenic micro-organisms may also be of interest for the study of infectious diseases.

It can be shown without objection that the formation of acetaldehyde is an intermediate product also in the case of butyric fermentation by *B. butyricus* (Fitz) when the fermentation is carried out in the presence of Na_2SO_3 . In place of butyl alcohol or butyric acid there is here formed ethyl alcohol and acetic acid in connection with the splitting processes, which, beside the gaseous decomposition products of sugar, could only lead to acetaldehyde production.

But it seems remarkable that also the fermentation of alcohol to acetic acid, which is technically used for acetic acid production, passes through the acetaldehyde stage:



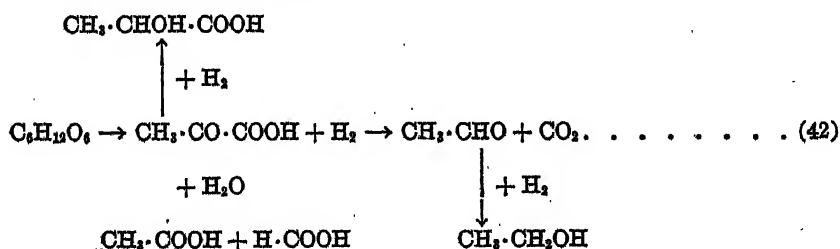
If the fermentations by yeast and bacteria discussed up to this point deal with intramolecular processes, with the splitting of compounds in which the inner shifting of oxygen plays a part, then in the acetic fermentation there is displayed an oxidative process in which, beside the Cannizzaro rearrangement, atmospheric oxygen also takes part. But the acetic fermentation is closely related to sugar splitting not only through the fact that many organisms can form acetic acid from carbohydrates but also in this, that in the oxidation of alcohol the substance which is formed by a definite agency is acted upon by another organism. Exactly as in the case of the previously described fixation of acetaldehyde as an intermediate product, so also in the case of acetic fermentation, especially by *B. ascendens* and *B. pasteurianum*, aldehyde, as the sulfite complex, accumulates in quite appreciable quantities. From one-third to three-fourths of the weight of acetic acid which is formed under the circumstances of the experiment, was obtained as acetaldehyde.

It was experienced that the fixation of acetaldehyde results much more satisfactorily in the presence of CaSO_3 than in the presence of Na_2SO_3 . The reason for this might be that the strongly alkaline alkali sulfite acts as a check on the agent causing the fermentation. Since the fixation of acetaldehyde can result only in accordance with an equilibrium—that in the case of acetic fermentation is displaced toward the side of dissociation—a part of the acetaldehyde is available for the normal process, i.e., for the further transformations. This can be obtained, however, according to the most recent results, not directly but under certain circumstances in the manner of a Cannizzaro reaction on the acetaldehyde. If acetic acid bacteria are given pure acetaldehyde or pyruvic acid they always produce from it equal molecular amounts of acetic acid and ethyl alcohol and this fact establishes a still closer parallelism between acetic fermentation and the alcoholic fermentation.

The appearance of acetaldehyde in the fermentation of sugar by *B. lactis aerogenes* or of pentoses by *B. acetoaethylicus* and others, likewise can also be demonstrated.

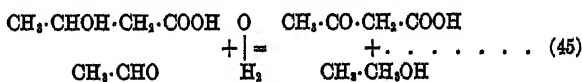
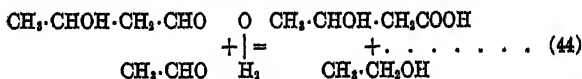
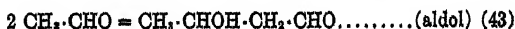
In this connection the recent work of Speakman is of interest. The work which he began in 1914–1915 interprets in a convincing manner the mechanism of the acetone fermentation. In using *B. acetoaethylicus* he was able to demonstrate that in the fermentation of glucose, maltose, or glycerol, pyruvic acid was the intermediate product which, by the way of acetaldehyde is the parent substance of the ethyl alcohol, as well as of the acetone.³

At the onset of the fermentation when practically no acetone is formed, the following formulas,



³ Compare also Freiberg, G. W., Proc. Soc. Exp. Biol. and Med., 23, 72 (1925).

and later when rapidity of the hydrogenation of the acetaldehyde is decreased in relation to its formation, the following scheme:



explain the situation, as the alcohol: acetone proportion of 2.2 is established, beginning with about the fifth day. The same explanation, however, is attempted, as in the fermentation by yeast, to explain the processes through a joint reaction of different carbonyl compounds (see page 59).

This review might give the impression that a central position may be ascribed to acetaldehyde in the processes summarized above. This view is not in general contradicted by the present state of our conceptions, which are supported in part by experimental data. Nevertheless it must not be overlooked that the final answer concerning the rôle of the parent substance of this aldehyde—pyruvic acid—in the course of alcoholic fermentation itself can only be obtained if in further investigations additional attention is given to the influence of factors that can be determined by physico-chemical methods.

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* Without claim for completeness. For detailed bibliography and further data see: Fuchs, W., *Samml. chem. und chem. techn. Vortr.*, 27, 1, (1922); Furness, R., *The Fermentation Industries*, London, (1924); Haeggund, E., *Samml. chem. und chem. techn. Vortr.*, 21, No. 4, (1914); Harden, A., *Alcoholic Fermentation*, London, (1923); Kopaczewski, W., *Catalyse et ses Applications*, Paris, (1925); Meyerhof, O., *Chemical Dynamics of Life Phenomena*, Philadelphia & London, (1924); Neuberg, C. and Hirsch, J., *Erg. Phys.*, 21, 400, (1923); Sørensen, S. P. L., *Proteins*, New York, (1925).

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THE RADIOACTIVITY OF THE ALKALI METALS

I. JOCELYN PATTON¹ WITH L. J. WALDBAUER²

The radioactivity of potassium and rubidium was first reported by Campbell and Wood (1) in 1906. Of the remaining alkali metals, lithium and sodium have been consistently reported inactive, while the activity of caesium is still doubtful (2). Numerous investigations have since been undertaken to determine the origin of the activity of potassium and rubidium, but the question is still undecided, as a careful scrutiny of the experimental evidence discloses many contradictions.

Three theories have been advanced to account for the radioactivity of the two alkali metals, viz.,

I. The activities of potassium and rubidium are due to a disintegration of the atoms of these elements;

II. A missing radioactive element of this series (atomic number 87) causes the apparent activity;

III. The beta rays emitted are not of radioactive origin, i.e., from the nucleus, but are comparable to the photo-electric effect.

The arguments that have been adduced in favor of I are:

a. Potassium and rubidium salts from various sources have an activity which is proportional to the percentage of potassium or rubidium present. The experimental evidence which has been brought forward in support of this argument is, however, exceedingly doubtful.

b. The beta rays emitted from potassium and rubidium are of different intensities and penetrating powers.

c. All attempts to isolate a new element from any potassium or rubidium salts have completely failed. A possible excep-

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tion may be found in Ebler's (3) "radioactive amalgam," which will be described later.

The principal objections to this theory are:

a. The number of beta rays emitted by either potassium or rubidium is very small, compared to the number emitted by any of the elements of the three well-known radioactive series. This objection is somewhat weakened by the findings of Hahn and Rothenbach (4) and Hoffmann (5), who found that the activity of metallic rubidium to metallic uranium is as 1:15.

b. The atomic numbers of the two "active" alkali elements are so low that it seems improbable that the nuclei of these elements actually break down due to their instability.

c. Caesium is not active, or only slightly so, yet its atomic number is considerably higher than either of the other alkali metals.

d. If potassium and rubidium are active, one would expect their disintegration products to be isotopic with calcium and strontium respectively, in accordance with the Soddy-Fajans disintegration law (loss of an electron from the nucleus shifts the element one group to the right in the Periodic Table.) No isotopes of calcium or strontium which could have been produced from the various isotopes of potassium and rubidium have been found.

In addition to the objections to the first theory, there is an argument in favor of the second theory, viz., that there is a missing element of atomic number 87, which is in all likelihood an alkali element. From its position between radon and radium, one would expect this missing element to be radioactive. It is a significant fact that the only group of elements containing an unknown member which, if existent, would be radioactive, is the only one whose other members show radioactivity. On the other hand, there is an objection to this theory too, in addition to the arguments in favor of the first, and that is that the missing element of Group I would be more nearly related to caesium than to either potassium or rubidium, and hence should be found associated with caesium rather than with either or both of the others. Nevertheless, the best-known caesium

mineral, pollux, has been consistently reported as ~~"inactive."~~

Objections (a) (b) and (d) to theory I might be considered as arguments in favor of theory III, while objection (c) would be an argument against this theory. This will be further discussed later.

In order to form a background for the discussion of the three theories, it will be necessary to give a fairly comprehensive review of all the literature dealing with the radioactivity of the alkali metals.

Campbell and Wood (1) found variations in the activity of various potassium salts (all derived from the Stassfurt deposits), but concluded that the discrepancies were due to the varying absorption of the rays from the bottom layers of material, due to the different densities of the salts. On attempting recrystallization of the salts, it was found that the activity of the mother liquor was 1 to 2 per cent lower than that of the crystals in some cases, and had the same activity in others. In a later paper, Campbell (6) states that he failed to observe or produce any difference in the activity of different samples of the same potassium compound. McLennan and Kennedy (7) examined a large number of compounds, and found that the activity varied from compound to compound, depending on the source. Unfortunately, these investigators assumed that the purity of all their compounds, even of those designated "commercial," was 100 per cent, and made their calculations on this basis. Had the percentage of potassium been determined for each sample, their results might have been of some value. In the case of the samples of potassium cyanide, analyses were made, and it is interesting to note that there actually was a variation in the activity of the potassium.

Quoting from McLennan and Kennedy's paper:

From the results set forth in the table, it will be seen that exceedingly wide variations were found in the potassium content of the various specimens. It will be seen too, that those salts which possessed the greater potassium content also exhibited the higher activities, and that, in the case of the more active, the radioactivities were approximately proportional to the potassium contents of the salts.

The results obtained with the potassium cyanide in this series of experiments, therefore, rather support the view taken by Campbell that the activities of potassium salts are directly proportional to the amount of potassium in them.

McLennan and Kennedy neglected to calculate the A/K values, which have been calculated by the present writers. It will be seen that the conclusions drawn in the lines just quoted are hardly justifiable. The sample exhibiting the highest activity had a percentage of potassium about one-seventh that of the next most active sample, and it is difficult to trace any proportionality whatever between the percentage of potassium and the radioactivity of the salt.

TABLE 1
Activity of potassium cyanide; McLennan and Kennedy (7)

SOURCE OF SALT	RELATIVE ACTIVITY "A"	PERCENTAGE OF K BY WEIGHT	$\frac{A}{K} \times 10^3$
Manufacturer A, No. 1.....	8.85	52.15	16.9
Manufacturer B, No. 1.....	1.40	9.44	14.8
Manufacturer C, No. 1.....	0.15	2.84	5.3
Manufacturer C, No. 2.....	0.10	2.76	3.6
Manufacturer C, No. 3.....	0.60	9.57	6.3
Manufacturer C, No. 4.....	0.00 (slight)	2.36	0.0
Manufacturer C, No. 5.....	1.47	7.64	19.3

Campbell (6) attributed the variation just mentioned to the fact that the ionization chamber used by McLennan and Kennedy was not protected by the usual aluminium foil, and to the additional fact that the change in the "natural ionization" of the air, due to the admission of fresh air while introducing the new sample, was so large as to preclude consistent results for the activity even of the same sample.

Biltz and Marcus (8) examined samples of potassium salts, extracted from minerals of widely different origins either by means of the hydrofluoric acid (9) or J. Lawrence Smith (10) method. They came to the conclusion that potassium salts from any source have an activity proportional to the potassium content of the salt. A close examination of their data fails to reveal any great constancy, as table 2 will show.

Biltz and Marcus make the interesting statement that, on precipitation of the sulfate sample obtained from spodumene as BaSO_4 , the activity of the sample disappeared. It seems strange that radium (to which they attribute the high activity) could have been carried along with the K_2SO_4 . It is a well-known fact that BaSO_4 has the property of adsorbing small

TABLE 2
Potassium sulphate; Biltz and Marcus (8)

SOURCE	% K	ACTIVITY	ACTIVITY/1%K
K_2SO_4 Kahlbaum	44.87	7.4	0.167
Carnallite (lying)	44.87	7.3	0.162
Rocksalt (Hartsalz)	44.2	7.1	0.160
Carnallite (hanging)	44.87	7.5	0.167
Orthoclase	27.0	4.35	0.161
Muscovite	32.7	5.4	0.165
Lepidolite	19.6	3.3	0.168
Leucite (Vesuvius)	27.4	4.8	0.175
Leucite (Albanian Mts.)	34.0	5.8	0.171
Spodumene (impure)	0.4	0.3	0.75
Spodumene (purified)	0.4	0.05	—calc. 0.125
Beryl (Norway)	7.0%K	1.0	(0.14)
trace Rb	5%Cs		

TABLE 3
Activities of the minerals examined; Biltz and Marcus

MINERAL	%K	ACTIVITY	ACTIVITY/1%K
Orthoclase	7.2	1.5	0.208
Muscovite	8.1	1.1	0.136
Lepidolite	6.3	1.1	0.175
Leucite (Vesuvius)	5.5	2.0	0.364
Leucite (Albanian Mts.)	8.7	1.9	0.218
Spodumene	0.12	1.5	12.5
Beryl (Norway)	0.07	0.0	—

amounts of substances as it precipitates, and it might easily have carried down some unknown active substance. It would be well to investigate spodumene again, and the writers have experiments on this mineral in view.

Henriot (11) has made the statement that his potassium chlo-

ride and sulfate samples had an activity proportional to the potassium content. He did try recrystallization, vaporization, etc., in an attempt to change the activities of the sample, but unsuccessfully. Elster and Geitel (12) tried fractional crystallization and fractional electrolysis, using a mercury cathode, but were also unsuccessful in changing the activity of the potassium chloride sample.

Strong (13), using the photographic method, found that some old samples of potassium compounds collected by Rowland were radioactive, but that potassium sulfate of recent manufacture was not nearly so active. Levin and Ruer (14), using the same method, found all samples of potassium salts active. Hoffmann (5), using a highly refined electrical method, found that the activity of potassium salts is proportional to the potassium content. Recently Harkins and Guy (15) measured the activities of potassium and rubidium salts and reached the same conclusion. Practically all of these investigators assumed the purity of their salts to be 100 per cent simply because the manufacturers claimed this purity.

Rubidium salts were first investigated by Campbell (16) who used the sulfate prepared by Kahlbaum, and found this active. McLennan and Kennedy (7) reported an exceedingly slight activity, but, as has been pointed out, their results are entirely untrustworthy. Strong (13), Levin and Ruer (14), and Büchner (17) all found rubidium salts active, using the photographic method. Henriot (11), Hahn and Rothenbach (4), Hoffmann (5), and Harkins and Guy (15) all verify this statement, using electrical methods. Hahn and Rothenbach measured the activities of several samples of rubidium salts of various ages, viz., one of recent date, prepared by Kahlbaum, one sample 11 years old, one 21 years old, and several rubidium alum samples, but found no variations in the activity. As they point out, rubidium is a rather difficult substance to work with, as there are no representative rubidium minerals, although the element is fairly widely scattered in nature.

All authors are agreed that lithium and sodium are possessed of no activity, although McLennan and Kennedy (7) did report

an active sample of rocksalt. Caesium has been reported as inactive by all authors but Zwaardemaker (2). He bases his conclusion on the fact that commercial caesium preparations produce physiological effects similar to those produced by well-known beta radiators. The effect is probably due to an active impurity. Ringer (18) states caesium to be inactive.

Campbell (1), from their absorption in tinfoil, concluded that the beta rays from potassium are heterogeneous. McLennan and Kennedy (7) reached a similar conclusion. Henriot (11) concluded that the beta rays from K_2SO_4 and from KCl are homogeneous, although the curve obtained by plotting the ionization current as ordinates against the weight of tinfoil used as abscissae did not give a simple exponential curve. By using a two-term exponential equation he found that the curve corresponded to two types of beta rays, the softer ones being 10 per cent of the total. In spite of his own experimental evidence, he concluded that the beta rays from potassium are homogeneous, and attributed the softer radiation to secondary rays emitted by the tinfoil screen. Harkins and Guy (15) apparently found the beta rays from potassium homogeneous.

Henriot (11), Hoffmann (5) and Harkins and Guy (15) found that the beta rays from rubidium are heterogeneous, Hahn and Rothenbach (4) apparently did not observe any heterogeneity, as they did not mention it. Rothenbach's papers were destroyed after his death, and thus most of the experimental results were lost before Hahn could publish them. Hoffmann gives 347 cm.^{-1} as the absorption coefficient of the harder rays in aluminium, and 900 cm.^{-1} for the softer radiation. Harkins and Guy state that the absorption coefficient decreases from 593 to 522 as the thickness of the aluminium foil increases from 0.0017 to 0.0051 cm.

Campbell (1) estimated that the rubidium rays are 16 times more absorbable than those of potassium, while Henriot (7) estimated them to be ten times more absorbable; Harkins and Guy (15) compromise by stating that the rubidium rays are from 10 to 15 times more absorbable than those of potassium. Hahn and Rothenbach (4) concluded that the activity of metal-

lic rubidium is to that of metallic uranium as 1:15; this was confirmed by Hoffmann (5) who found the ratio 1:14. Harkins and Guy (15) state that the activities of rubidium to potassium are in the ratio of 1.39:1.00.

On discovering the heterogeneity of the beta rays from potassium, Campbell (1) attempted to isolate a new element from the potassium salts by means of fractional crystallization, heating in the blowpipe flame, and by electrolysis. In some cases the crystals were more active than the mother liquor by 1 or 2 per cent, but not consistently. If he expected to find a new alkali element, one would expect its salts to be more soluble in water than either KCl or K_2SO_4 . Campbell gave up the attempt to fractionate the potassium salts, simply because of the experimental difficulties involved.

Elster and Geitel (19) and Henriot (11) tried fractional crystallization, but also without results. The former investigators also tried fractional electrolysis of KCl using a mercury cathode, but found no difference in the activities of the fractions. It was impossible to crystallize fractionally rubidium salts, because of the small quantities available.

Ebler (3) investigated the sediment from the Max Spring at Dürkheim, Germany, and found it very highly active. At this point, it might be of interest to quote rather fully from his paper:

This particular spring is the one in whose water rubidium and caesium were first discovered, hence one might hope to find the sixth alkali metal here.

The sediment from the spring is chiefly remarkable for its high radioactivity, its high arsenic content, and the large quantity of it. The spring deposits 20 kgm. of sediment every twenty-four hours.

After solution of the sediment in aqua regia, and removal of all of the elements up to the alkaline earths, it is found that the mother liquor is highly active. On further removal of the alkaline earths, it is found that these are inactive; in other words, the active substance is in the solution which now contains only the alkali metals. In attempting to concentrate the solution of the alkali metals, it was found that the crystals last obtained showed only a weak activity, i.e., much weaker than the original mother liquor would lead one to expect.

When several kilogrammes of the aqueous solution, acidified with hydrochloric acid, were steam-distilled, the distillate was very strongly active, while the steam distilled solution showed only a slight activity. On standing, the latter regained almost all of its former activity, and the former decreased in activity but did not entirely lose it. A portion of the radioactive substance was evidently volatile with steam. A quantitative test showed that the amount by which the mother liquor decreased in activity (from its original value) was equal to the activity of the distillate.

Electrolysis of the radioactive distillate, using a mercury cathode, caused the mercury to become radioactive. On washing the amalgam with water, the mercury became inactive, and the wash-water highly active. When some of the active amalgam was covered with a saturated solution of caesium chloride, it was found that the caesium had formed an amalgam with the mercury. (Care was taken that no CsCl solution adhered to the mercury.) A blank test with ordinary mercury (inactive) showed no trace of a Cs amalgam. Thus the active substance displaced caesium from a salt, yet caesium is the most electropositive of all metals. This could only occur if the unknown metal were more electropositive than caesium.

Practically all of the investigators since the publication of Ebler's paper have dismissed it with a line or two to the effect that Ebler must have been mistaken. It would seem a relatively simple matter either to corroborate or to refute Ebler's claims, yet no one has taken the trouble to do so.

T. W. Richards and Archibald (20) reported that, in the purification of caesium salts (which were the purest obtainable) for atomic weight determinations, no trace of the missing alkali element was observed. Baxter (21) examined pollux (the commonest caesium mineral) from Paris, Maine, for the missing element, but failed to find it. Dennis and Wyckoff (22) examined various fractions of the alkali chlorides obtained from pollux (from Auburn, Main) spectrographically in the red, visible and the ultra-violet regions, but failed to find any trace of the missing alkali element.

Recently, Druce and Loring (28) have laid claim to the discovery of several of the missing elements, including number 87. They prepared impure rhenium oxide from commercial

manganese sulfate, and used this in the usual X-ray method. To quote from their paper: "The method employed consisted in exciting the characteristic X-radiations of the elements present in the sample, spreading these into a spectrum by the Bragg method of reflection at a crystal plane, and recording the lines photographically." Later, they claim to have isolated more of 87 from a sample of pyrolusite, and found the chemical properties of this element resembling the expected ones. Just what the details were, is not stated. Under "Current Topics and Events" in the December 26 issue of *Nature*, a contributor questions the validity of the claims made by Druce and Loring, and points out a number of discrepancies in their statements. The only conclusion to be drawn at the present writing is that the identification of element number 87 is still very doubtful. Certainly nothing definite is known about its radioactive properties.

Zwaardemaker (2) believes the activity of caesium to be due to a higher member of the alkali metal group. Hoffmann (5) investigated pollux and found it inactive. It might be mentioned that Hoffmann's work is extremely accurate, as he eliminated a great many of the usual sources of error in making his measurements.

Lachs (23) was the first to point out that, if potassium and rubidium are radioactive, their disintegration products must be isotopic with calcium and strontium respectively, in accordance with the Soddy-Fajans disintegration law. Hahn and Rothenbach (4) calculated that the half-life of rubidium is 10^{11} years, and that of potassium about three times as great. On this basis, it would be very difficult to detect any calcium isotope of atomic weight 39 or 41, even in potassium minerals of the greatest age. It would be somewhat simpler to detect the strontium isotope of atomic weight 85 or 87, but unfortunately we have no rubidium minerals as such, the rubidium being fairly well scattered. Kossel (24) suggested that only one of the potassium isotopes might be radioactive, and suggested the one of atomic weight 43. As Harkins and Guy (15) point out, no isotope of that atomic weight has been found to exist. Har-

kins and Guy, on the basis of Harkins' studies on isotopic numbers, are led to believe that the isotope of potassium of atomic weight 40 or 41 is responsible. (The isotope of atomic weight 40 has not been found in positive ray work.) Up to the present only the two isotopes of calcium, atomic weights 40 and 44, have been found by Aston (25); and only two of strontium, 88 and 86 (in the order of their intensities). No systematic investigation of the calcium content of potassium minerals by means of the mass-spectrograph has been published, if, indeed, an investigation of this sort has ever been undertaken.

The objection to the theory that the presence of the sixth alkali metal causes the activity of potassium and rubidium, viz., that one would expect this unknown alkali metal to be associated with caesium, is somewhat weakened by Ebler's findings. The objection is strengthened by the findings of Richards and Archibald, Baxter, and Dennis and Wyckoff.

The objections to the theory that potassium and rubidium are intrinsically active, might be taken as arguments in favor of the theory that the emission of photo-electrons is the cause of the apparent "radioactivity" of these elements. The fact that caesium, the most electro-positive of all of the known elements, is not active in pollux argues against this, as does the fact that lithium and sodium have consistently been reported as "inactive." The photo-electric effect places the alkali metals in the series $Cs > Rb > K > Na > Li$, whereas the radioactivity series is $Rb > K > Cs > Na > Li$. Furthermore, while studying the photographic effect of potassium and rubidium salts, Büchner (17) found that the plates darkened even when Rb_2SO_4 was dissolved in water, and evaporated to dryness in the dark. Later he kept some of his samples in the dark for six months, and found them still "active." This seems conclusive evidence against the "photo-electric" theory of activity.

None of the alkali metals has been found to emit alpha particles although R. J. Strutt (26) found that a number of beryls contain helium far in excess of that expected from the content of radium or thorium compounds. Piutti (27) examined 26 beryls spectroscopically, and found the helium spectrum well-defined

in all of them, although not all of the samples were radioactive. The helium does not seem traceable to the alkali metals, or at least, alpha rays, if emitted by them, are extremely slow, and have not, as yet, been detected.

SUMMARY

From the above review of the literature on the radioactivity of the alkali metals, the following are the only definite conclusions that may be drawn:

- a. Lithium and sodium do not show any radioactivity.
- b. Potassium and rubidium salts emit heterogeneous beta rays.

- c. No alkali metal emits detectable alpha particles.

The following questions still remain unanswered:

- a. What are the absorption coefficients in aluminium of the two beta radiations from potassium?
- b. What disintegrations cause the heterogeneity of the rays from potassium and rubidium?
- c. Is caesium active?
- d. Can a sixth alkali metal be isolated from the salts of the others? Is it radioactive?
- e. If the sixth alkali element cannot be discovered, do isotopes of calcium of atomic weights 39 and 41, and isotopes of strontium with atomic weights of 85 and 87, exist?
- f. What is the source of the helium in the beryls?

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SUBSTANCES WHICH AFFECT PHOTOGRAPHIC PLATES IN THE DARK

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The effect which some substances have on photographic plates in the dark has been studied with the idea of devising a method for identifying the ingredients in certain mixtures often presented for microchemical analysis.

HISTORICAL REVIEW

In 1842 Moser (1) noticed that when certain bodies were brought in contact with a silver iodide plate in the dark the developed plate showed an image, the silver iodide being darkened most where the action of the body was strongest. He concluded that all bodies may possibly be considered "self-luminous."

In 1857 and 1859 Niepce de Saint Victor (2, 3) recognized the effect produced on silver bromide paper by certain substances, such as paper, marble, chalk, feathers, and cotton, when they had been exposed to light before their contact with the sensitive paper. He found that this action would pass through a layer of air 1 cm. thick and could also penetrate collodion and gelatin but was stopped by glass, minerals, and certain varnishes. He believed that the light "was absorbed and conserved" by these bodies to be sent out later in the form of radiations.

Apparently the subject was not studied further for thirty-eight years. In 1896 Colson (4, 5) found that zinc, cadmium, and magnesium affected the photographic plate in the dark. He described this phenomenon to the emanation of metallic vapors. Tin, iron, copper, and lead were found to be inactive.

Pellat (6) in 1896 and Thomson (10) in 1897 agreed with Colson that vapors given off from the metallic surfaces were responsi-

ble for the effects obtained. In order to confirm this assumption, Thomson passed an air-blast between the sensitive surface and the metal. This produced a distortion of the image.

In 1896 MacKay (7) observed the darkening of a photographic plate when it was brought into a strong electrical field with an iron object on its surface. He ascribed this effect to magnetism. At the same time Packer (9) found that a metal became active toward the photographic film on exposure to light only when it had not previously come in contact with a conductor of electricity.

Arnold's account (13) published in 1897, showed that the sulfides of zinc, calcium, and barium, as well as uranium and calcium tungstates and retene exhibited photographic activity in the dark. He attributed the action of retene to Becquerel rays, but observed that the closely related substances, anthracene, anthraquinone, and phenanthrene, did not have this property. He apparently considered the activity in general to be due to some sort of radiation.

Russell began his classic work on this subject in 1897 (14). He observed that when a zinc screen was placed between a uranium compound and the photographically sensitive surface, the silver was acted upon not only by the radiations from the uranium but also by the zinc screen. This led him to make direct tests with zinc, which showed that polished zinc was more active than zinc which had been exposed to the air for some time. A glass plate interposed between the zinc and the sensitive surface stopped this action, but many substances, such as celluloid, sheet gelatin, collodion, vegetable parchment, gold beater's skin, and tracing paper, permitted its transmission. Aqueous vapor was found not to be an active agent in producing these effects. The action took place in an atmosphere of hydrogen as it did in air. Under ordinary conditions results were obtained in an atmosphere of carbon dioxide, although Russell believed that this effect was more probably due to the action of the carbon dioxide on the zinc plate. Alteration of the temperature appeared to produce marked differences in the intensity of the picture obtained, an increase in temperature greatly augmenting

the activity of the zinc. A piece of mahogany 3.5 mm. thick, which had been in this form for at least 35 years, gave, after a week's exposure, a good picture. Coal, coke, sulphur, and sugar, however, exerted no action.

In 1898, Russell (15) published the results of additional experiments on a large number of substances, both organic and inorganic, conducted principally with a view to determining the active agent concerned in the darkening of the photographic plate in the absence of light. As a result of these tests he concluded "that certain metals have the property of giving off, even at ordinary temperatures, vapor which affects a sensitive photographic plate, that this vapor can be carried along by a current of air, and that it has the power of passing through thin sheets of such bodies as gelatin, celluloid, collodion. . . ."

Lengyel (16), Tucker (17), Bothamley (18), Sperber (19), and Vincent (20) reported the results of their work in 1898 and 1899. Lengyel's conception was that hydrogen, formed in the presence of moisture on the metallic surface, might be considered the active agent. If the conditions for the formation of hydrogen were not present, the activity was absent. He considered it reasonable to conclude that the activity was produced either by a vapor emitted by the metal or by some sort of radiation. Like Colson, Tucker had observed the action of printer's ink on the plate. Sperber repeated some of Russell's experiments for the purpose of determining whether the activity was due to a vapor generated by the substance or to active rays. He concluded that the active substance produced vibrations similar to the vibrations of ultra-violet rays and, like them, chemically active. Bothamley observed the breaking-down of the latent image through the long action of turpentine and similar substances, which formed hydrogen peroxide when they oxidized in the air.

Russell (21), in 1899, confirmed his previous findings, and showed that the action of various bodies on a photographic plate in the dark was probably due to the presence of hydrogen peroxide, which appeared to be formed when the metal sheets were oxidized in moist air. On the basis of this assumption,

Russell inferred that on supplying to the metallic surface more moisture than it would obtain from the atmosphere and the photographic plate, the activity would be increased. This was found to be the case. He made many tests on essential oils, which proved to be active, also the ordinary vegetable oils, such as linseed, colza, and olive. The last two oils were not as active as linseed oil. The mineral oils, on the contrary, were inactive, as were also such compounds as benzene, phenol, naphthalene, acetaldehyde, methyl alcohol, and "coal naphtha." Vincent (20) practically substantiated the observations of Russell in 1899 and concluded that the active substance must be hydrogen peroxide.

Levison (22), in 1900, found that Canada balsam, when baked hard and placed on the sensitive side of the photographic plate or separated from it by a layer of black paper, affected the plate very much as light would. In his opinion this effect could be attributed to true Becquerel rays, as it passed through the black paper, which is impervious to ordinary light.

About two years later Vignon (23) obtained pictures of coins which he had rubbed with zinc dust and exposed to the photographic plate. This effect, of course, was due to the zinc, which Russell had found to be active.

According to Villard (24), certain inorganic substances acquired under the influence of ozone an ability to act in this manner which persisted for more than a day. Bismuth and aluminium after treatment with ozone gave images, but the photographic impression was not uniform. It consisted of a series of black points on a gray background.

Graetz's tests (25, 31), reported in 1902 and 1904, demonstrated that the action of hydrogen peroxide on the photographic plate would pass through gold leaf and aluminium foil. Like Thomson, Graetz had observed that when a current of air was blown through the hydrogen peroxide vapor the photographic effect still persisted and was registered, as shown on developing, in the form of intense black points on the plate. He assumed that particles of an unknown nature must have bombarded the plate.

Bogojawlensky (28), in 1903, obtained positive results on

several metals. He concluded that Russell's explanation of the phenomenon was plausible.

Schweidler (29), the same year, attempted to prove that retene possessed ionizing action, but failed to do so.

In 1903, Dony-Henault (30) confirmed Russell's tests with respect to hydrogen peroxide and satisfied himself that bodies indifferent to the photographic plate became active after being exposed to the action of hydrogen peroxide.

Blaas and Czermak (32), in 1904, again confirmed the findings of Russell. They observed that paper, wood, shellac, leather, silk, and other substances affected the plate after they had been exposed to light, and proposed designating such substances which became active after insolation as "photechisch" (light retaining) and the property itself as "photechie." These photo-active bodies, they concluded, sent out a diffuse radiation which belonged to the blue end of the spectrum.

Kufferath and Merckens (33), in 1904, and Merckens alone, in 1905 (40), obtained photographic images with magnesium, aluminium, zinc, cadmium, nickel, cobalt, and lead. No image was obtained with tin. In a vacuum, magnesium produced no effect, even when in contact with the plate. These investigators explained the darkening of the plate by the metals on the basis that the oxidation of the active metal produced hydrogen peroxide, which acted on the plate. They considered this to be due to the fact that upon its decomposition active oxygen was formed, which, in a nascent condition, changed the silver bromide of the gelatin layer to an easily reducible oxide.

Van Aubel (34), in 1904, found that colophony was active through black paper but not through a sheet of copper. Abietic acid, copal resin, and mastic resin gave similar results but in a different degree. Heating the colophony appeared to drive off the active material.

According to Schaum and Braun (35), the action of ozone on the photographic plate could be explained as a purely chemical reaction, with the formation of a silver peroxide, oxybromides, etc., or an ionic action, with the discharge of silver ions. The action of hydrogen peroxide might be explained in much the same manner.

Stöckert's test (36), in 1904, as well as those of Precht and Otsuki (38), in 1905, practically confirmed the findings of Graetz. Precht and Otsuki observed that the reaction between hydrogen peroxide and silver bromide gelatin depended largely on the temperature and that the photographic activity increased with the concentration to a certain maximum, after which it decreased.

In 1905 Campbell (41) reported the results of experiments on the influence producing "spontaneous ionization" in a closed vessel containing gas. He stated that the walls of the vessel were responsible for an ionization effect which he had been able to measure. He concluded that any such ionization in a closed vessel containing gas might be attributed to a radiation proceeding from the walls, that certain substances, such as tin, zinc, graphite, and platinum, might produce part of these radiations, analogous to the secondary radiations excited by Röntgen and other rays. Campbell suggested that all elements are radioactive to some degree and that some might be expected to give off an emanation. All efforts directly to detect such an emanation from lead, zinc, and aluminium, have failed, however.

The experiments of Kahlbaum and Steffens (42), in 1905, appear to indicate that gravity has some influence on the photographic activity of many substances. Their work showed that the action produced from the lower side of a metallic plate, as recorded by the sensitive layer after developing, was more pronounced than that produced by the upper side.

Streintz and Strohschneider (43) confirming the results obtained by earlier workers, reported in 1905 that they had found magnesium, aluminium, zinc, and cadmium to possess the property, after polishing, of producing an image on paper impregnated with potassium iodide. They believed that the decomposition of the potassium iodide was due to "the electrolytic solution-pressure in the neighborhood of the surrounding ions." Since they could detect the effect, even if the metal and paper were separated by a thin layer of air, they concluded that the ionization passed through the air. The effect obtained, they stated, had the character of a radiation and should be designated "metallic radiation."

Melander (44) was of the opinion (1905) that at ordinary temperatures all metals send out violet and ultra-violet rays, but that the rays are so weak that they can not be detected with the eye. Nevertheless, the ionizing action of these rays plays an important part in the phenomenon recorded on the photographic plate, when they strike its sensitive surface.

The work reported by Baborevsky and Vojtech (46), in 1906, demonstrated that ammonium amalgam did not emit any photographically active rays.

Campbell's (45) new tests in 1906 proved to his satisfaction that the emission of ionizing radiations was an inherent property of all the metals investigated (lead, copper, aluminium, zinc, iron, platinum, tin, silver, and gold). Although there seemed to be no reason why this assumption should not be extended to all substances, it did not follow that ray-emission should necessarily be identified with radioactivity in the sense that it signified accompanying atomic change.

Piltschikoff (48), in 1906, recognized the so-called radiations which some metals produced as "Moser rays," named in honor of the physicist who discovered them (1). He found that these rays could be deflected by a stream of air, but not by a magnetic or electrical field.

In 1906 Russell (47) reported the results of new tests, which were confined largely to the action of plant substances on the photographic plate in the dark. Leaves, seeds, roots, bulbs, and, in fact, practically all vegetable substances acted in the same way. In many instances the tested material was subjected to considerable pressure before being brought in contact with the photographic plate in the dark. As a working hypothesis Russell reaffirmed the fact that the active material in these substances was hydrogen peroxide. He called attention to the fact that this action on the photographic plate by such substances is naturally what would be expected to occur, supposing, as has been stated by Usher and Priestley (37), that hydrogen peroxide and formaldehyde are the first products of plant growth.

Dombrowsky's dissertation (49), in 1908, covered the literature of this subject thoroughly. Glass, quartz, paraffin, silver,

gold coin, platinum, and palladium were left in contact with a photographic plate for two days. No action was evident on development. On the other hand, zinc, aluminium, and cadmium, when left for a similar period in contact with the plate, produced dark spots on the sensitive film when the plate was developed. Bright spots on the metals caused very intensive darkening. Tests were also made to avoid direct contact by placing the metals from 2 to 5 mm. from the plate; in these the active substances also had an effect. When a metal sheet was caused to act on the photo-sensitive surface at different distances, the darkening of the plate decreased with the distance and became diffused. Various screens interposed between the active materials and the plate had different effects. Substances like celluloid, gelatin, and collodion permitted the transmission of the activity. Completely dried gelatin, however, was practically impermeable. Activity at higher temperatures was more intensive than that at lower temperatures. Dombrowsky's experiments showed that a metal sheet exposed to hydrogen peroxide vapor adsorbed a little of it and that there was a slow chemical reaction between the hydrogen peroxide and the silver bromide-gelatin film. The final blackening of the plate on development was the result of both of these processes. This investigator found that the metals alone were not active, except in the presence of moisture and oxygen.

Elizabeth Legrady (50), in 1908, was unable to obtain any darkening of the photographic plate by cadmium, copper, aluminium, magnesium, and zinc, when exposed in an atmosphere of hydrogen, nitrogen, oxygen, or air, either dry or moist. As a result of her tests she was led to the conclusion that these metals in themselves do not exert any action on the photographic plate, and do not form hydrogen peroxide or any radiation capable of producing an image. She considered the effect reported by others to be due to an ionizing action, the resultant image being produced by ionized hydrogen.

Saeland (51), in 1909, observed that if immediately after polishing, metals (Mg, Zn, Al, Pb) were placed in vacuum, dry air, or hydrogen, they caused practically no blackening on the

photographic plate in the dark. His tests led him to conclude that such action is due entirely to the chemical action of the hydrogen peroxide formed, and therefore could not be due to a radiation.

In 1908, Russell (52) reported that the action of ordinary resin on the photographic plate was rather slow, exposure from two to three days being necessary to obtain a good picture. "Amber resin," however, was more active. Increasing the temperature augmented the activity, so that an exposure of only from three to four hours was necessary. Abietic acid, an important constituent of resin, gave a distinct image after exposure for eighteen hours. The presence of oxygen appeared to be necessary for this action. Russell believed that the action of resins and related bodies was produced by a vapor rather than by any form of radio-activity.

The work of O. and A. Dony-Henault in 1908 (53) convinced them that the photographic action of a solution of hydrogen peroxide on a silver bromide-gelatin plate was not due to radio-activity, but to the effect produced by the hydrogen peroxide existing as a vapor.

Brooks (54), in 1909 called attention to the fact that the substances tested by Russell and others owed their activity to their ability to form organic peroxides by autoxidation. Freer and Novy (26) and Clover and Richmond (27) had previously shown that organic peroxides were slowly hydrolyzed by water, with the formation of hydrogen peroxide.

Ebler (55), in 1909, explained the action of metals on the photographic plate on the supposition that the metal becomes oxidized by atmospheric oxygen, a higher oxide being formed (with carbon dioxide in the air), finally producing hydrogen peroxide, moisture also being present. He found that zinc and many other metals behaved in this manner. According to his conclusions, aside from the metallic surface, oxygen and a moist atmosphere were necessary to produce the effect observed.

For the next fourteen years, little work, if any, appears to have been done in this field. Rumpf (56), in 1923, from his experiments with zinc showed that its action on a photographic

plate can not be due to a wave radiation or to electrons but must be due to a chemical reaction caused by oxidation by very small quantities of hydrogen peroxide.

The investigations of Sheppard and Wightman (57), in 1923, indicated that it is very unlikely that hydrogen peroxide acts as a reducing agent on silver halide. They believed that the silver halide was affected by the energy evolved primarily as a chemi-luminescence of short wave-length, this energy being the result of the decomposition of hydrogen peroxide which takes place in the presence of catalysts. The work of Wightman, Trivelli and Sheppard (60), in 1925, showed that acid hydrogen peroxide is slightly more effective than alkaline peroxide in producing an image.

Baughman and Jamieson (59) have shown that freshly expressed oils and fats possess no activity towards the photographic plate until they have been exposed to the sun for several hours. The saturated fatty acids were faintly active before exposure to the sun, becoming extremely active after exposure. Heating to approximately 120° for several hours appeared to destroy or weaken this activity. These results seemed to them to confirm Russell's view that the active substance is hydrogen peroxide.

Kugelmass and McQuarrie (61) came to the conclusion that ultraviolet light is not emitted by cod liver oil and certain other substances prescribed for rickets when they are oxidized in alkaline media. The darkening effect produced on the photographic plate by such substances is held to be due to reducing vapors, an effect comparable to that obtained by Russell and others with hydrogen peroxide.

Stutz, Nelson, and Schmutz (62) have recently found that a 0.01 per cent solution of hydrogen peroxide in water produces an image comparable to the effect produced by linseed oil after it had been exposed to the light for an hour. In the case of drying oils, it was indicated that a rapid reaction took place, confined chiefly to the surface, resulting in the formation of a skin which was relatively impervious to hydrogen peroxide. When this skin was broken, hydrogen peroxide was again actively evolved. They conceded that the primary oxidation of an oil was proba-

bly due to a molecular autoxidation, where molecules of oxygen were added at the double linkings, thus forming peroxides. Their work is being continued.

The foregoing résumé of the literature shows that the effect of various metals and organic substances upon photographic plates in the dark has been extensively studied since its discovery by Moser (1) in 1842, and that many explanations have been proposed to account for the effects. Some workers, especially the earlier ones, consider the phenomenon to be due to radiations of some sort. The majority of students, however, believe that they are produced by an emanation, hydrogen peroxide being most frequently considered as the active agent.

EXPERIMENTAL WORK

The principal part of the experimental work was done on plant substances and other organic material. A large variety of plant materials were tested. Some of them were used in the powdered form and others, such as leaves and flowers, after they had been placed in a plant press and then laid directly against the photographic emulsion. Empty desiccator jars covered with black paper and kept in a dark room were used for the tests. In some instances the material was placed directly on the photographic plate; in others it was placed in a shallow dish and the photographic plate was allowed to rest upon the dish, emulsion side down, with an interposed mask of cardboard or other inactive material containing some distinctive cut-out figure. The periods of exposure ranged from 2 to approximately 16 hours. Room temperature (22° to 24°) was usually maintained. After the test the photographic plate was developed and fixed according to the usual methods.

In order to make sure that the experimental procedure described was adapted for bringing out the phenomena under investigation, tests were made on some of the metals found by earlier workers to be active—zinc, magnesium, cadmium, and aluminium. All of these produced images on the plate, confirming the findings of previous workers.

TESTS ON PLANT MATERIAL

Many plant products affected the photographic film in the dark. Ground nutmeg produced a slight effect at a room temperature of 22° and a much more marked effect after an exposure for 16 hours at 46°. Ground cinnamon behaved similarly, a distinct picture being obtained after a 4-hour exposure at 45°. Grapefruit and orange skin gave an image only after being exposed to the plate for 16 hours at room temperature. Sandalwood (*Santalum album*) showed an effect after exposure for 2 hours at 45°, but practically none after an exposure for 16 hours at room temperature. Powdered anise seed and cardamon berries gave no definite results.

Orange leaves, lemon leaves, orange blossoms, grapefruit leaves, oak leaves, and maple leaves were first dried by placing them between blotting paper and subjecting them to moderate pressure. At room and higher temperatures all gave an image which in the early stages of development of the plate was distinctly visible, but which was so weak that prolonged development, in an effort to make it intense enough for reproduction, actually resulted in its disappearance.

Certain plant materials were inactive toward the photographic plate in the dark at either 22° or 45° and previous exposure to sunlight for several hours did not appear to produce any activity in them. The following substances behaved in this manner: Lavender flowers, celery seed, coriander fruit, fennel fruit, ground mace, asafoetida, fenugreek, sassafras bark, camphor gum, ground mustard, turmeric (powdered), corn starch, rice starch, potato starch, wheat flour, rye flour, barley flour, and white corn meal.

TESTS ON OILS

Many of the essential oils and allied substances gave distinct images on the photographic plate. Lemon oil, oil of citronella, citral, oil of peppermint, and cedarwood oil gave distinct reactions after exposure for 16 hours at room temperature (24°). Pine oil and oil of eucalyptus showed no reaction after exposure for 16 hours at room temperature, but were distinctly active after an exposure for 4 hours at 50°, and 52°

TESTS ON MISCELLANEOUS SUBSTANCES

Wood turpentine, abietic acid, and powdered rosin gave pictures on developing after an exposure for 16 hours to the photographic plate in the dark at 22°. Benzaldehyde also gave a distinct reaction under the same conditions. No reaction was obtained with the following substances, either before or after exposure to the sunlight for a considerable time: Menthol, vanillin, pyridine, nicotine, carbon bisulphide, ether, and naphthalene. It is interesting to note, in this connection, that aromatic principles gave the most striking results, particularly at 45° or slightly higher temperatures.

The pictures obtained with plant material strikingly resembled those obtained with hydrogen peroxide. Sheets of filter paper impregnated with titanium sulphate solution, which is often recommended for determining hydrogen peroxide, were placed over the tested material. Where these sheets rested on the edge of the petri-dish containing the active material they took on a yellow-orange color, suggesting that the active substance is actually hydrogen peroxide.

DISCUSSION

Some of the early workers assumed that the effects obtained on a photographic plate in the dark were due to radiations proceeding from the active substance. As early as 1857, however, Niepce de Saint Victor had recognized that the influence responsible for the darkening of the plate would not pass through glass, and more recently Russell and others have shown that sheets of mica and other minerals are impervious. If radiations were sent out from the tested substances, they should have been able to penetrate glass and mica. On the other hand, cardboard, black paper and gelatine, through which the plate is affected would be capable of transmitting volatile or vaporous substances. Moreover, no positive demonstration of the nature of the supposed radiations has ever been given. Accordingly, the alternative view that emanations from the active substances produce the observed effects seems more plausible. That emana-

tions are actually responsible is clearly shown by the following experiments:

a. Russell, Vincent, and others, have proved conclusively that the effect in question would penetrate all sorts of porous materials but not a single impervious screen.

b. Thomson found that the interposition of an air-blast between the active material and the photographic plate caused the image to elongate, showing that the effect is due to a stream of vapor capable of being deflected mechanically.

c. The writer found that an increase of temperature intensified the results obtained with certain plant materials, such as sandalwood. Almost without exception, these are known to contain volatile aromatic principles, the evolution of which would be accelerated by heating.

d. Hydrogen peroxide, even in very dilute solutions, produced effects indistinguishable from those obtained with various active substances. Glass and mica, of course, are impervious to its passage, but actual experiments show that cardboard and black paper screens allow it to penetrate readily.

e. Tests with titanium sulfate paper made by Russell and others, including the writer, gave the same results with several of the active substances as with hydrogen peroxide.

f. As shown by Lengyel, Russell, and Dombrowsky, both oxygen and moisture must be present to produce a darkening of the plate. As hydrogen peroxide could not be produced in their absence, the view that this compound is the active agent seems inescapable.

g. Admittedly, these considerations do not prove that hydrogen peroxide is the only substance that is active. As has been suggested by Brooks, organic peroxides may also be present. However, it seems simpler to assume that hydrogen peroxide is the active substance in all cases.

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CATALYSIS AND AUTO-OXIDATION

ANTI-OXYGENIC AND PRO-OXYGENIC ACTIVITY

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INTRODUCTION

Oxygen is the most abundant element in the crust of the earth, of which it constitutes nearly half of the mass. In a free state it forms an essential part of the atmosphere, and due to its great reactivity it tends to act upon the substances with which it comes in contact. It is therefore quite natural to find the study of this element, by Lavoisier, at the very beginning of the science of chemistry.

¹ Translated from the French by John R. Johnson, University of Illinois, Urbana, Illinois.

This element plays a fundamental rôle in nature, and among natural phenomena there is none of greater importance than spontaneous oxidation by free oxygen, commonly known as auto-oxidation. Respiration, the essence of life itself, might be conceded to fall into the domain of auto-oxidation.

Oxygen may act upon living organisms or inanimate objects in a multitude of ways. According to the need of man, its effects may sometimes be useful, sometimes harmful. Thus, man may wish to favor or to deter its action, and for this purpose a number of instruments have been employed, especially catalytic influences. Among the latter (the only ones with which we shall be concerned) we have called anti-oxygenic activity (negative catalysis), that which inhibits the action of oxygen, and pro-oxygenic activity (positive catalysis), that which accelerates it.

1. RÉSUMÉ OF EXPERIMENTAL OBSERVATIONS

I. HISTORICAL REVIEW

It would probably be possible to trace the employment of anti-oxygens and of pro-oxygens to the most ancient times. One would find among the various recipes and formulas which have been handed down from the old alchemists, certain ones which have for their purpose the exaltation or inhibition of the action of oxygen. The discussion which follows will be concerned only with observations resulting from the use of scientific methods. Even thus restricted, the task of preparing a complete bibliography of this subject is exceedingly difficult. We have limited our field to anti-oxygenic catalysis, of which very little discussion is found in the general treatises on catalysis, and refer for pro-oxygenic catalysis to the general works in this field.²

Phosphorus. Berthollet in 1797 made the curious observation that traces of the vapors of sulfur compounds prevented the luminescence of phosphorus in a dilute atmosphere of oxygen. This is the first observation of anti-oxygenic action that has been

² Among the works which treat of the historical aspect of catalysis may be cited: Paul Sabatier, "Catalysis in Organic Chemistry," transl. by E. Emmet Reid. D. Van Nostrand Co., New York, 1922.

found.³ This phenomenon was studied later by a number of investigators.⁴

Sulfur compounds. The oxidation of phosphorus and its prevention are very closely related to the recent observations of Delepine, who showed that traces of the vapor of ether, acetaldehyde, and other substances, hinder the luminescence of certain sulfur compounds (methyl dithiocarbonate, etc.).⁵

Explosive gas mixtures. The researches of Davy⁶ in 1817 and of other investigators⁷ have shown that the detonation of an explosive mixture of hydrogen and oxygen (two volumes of hydrogen and one volume of oxygen), or the slow combustion of hydrogen under the influence of platinum, is prevented by the presence of relatively small amounts of certain gases, such as ethylene and carbon monoxide.

Oxidizable gases or vapors. Observations analogous to those cited above have been made with other gases and inflammable vapors. Thus, Frankland observed that ethylene diminished markedly the inflammability of carbon disulfide vapor.⁸

Chloroform. Rump in 1868 was the first to report that traces of alcohol protected chloroform against atmospheric oxidation.⁹ This observation has served as the basis of a practical method for the stabilization of anesthetic chloroform and has resulted in lessening the dangers attendant upon its use in surgical operations.

Sodium sulfite. Auto-oxidation of this substance and means of preventing it have been the object of many important studies.

³ Journ. de l'Ecole polytechn., series 3, 277 (1797).

⁴ It would be out of place to give here a detailed bibliography of this special topic. The following is a list of the principal workers in this field: Thenard, Graham, Schoenbein, Joubert, Centnerszwer, Scharff, Thorne and Tutton, Vant' Hoff, Jungfleisch, Jorissen, Lord Rayleigh.

⁵ Bull. soc. chim. (4) 31, 762 (1922).

⁶ Trans. Roy. Soc. London, 1817, 45; Ann. chim. phys. (2) 4, 347 (1817).

⁷ W. Henry, Edw. Turner, Faraday, Graham, etc., and more recently Tanatar, Jorissen.

⁸ E. Frankland, Chem. News, 6, 3 (1862); see also the work of Turpain (1890) and of Dixon (1925).

⁹ "Ueber die Prüfung des Chloroforms," Hanover, 1868. The stabilization of chloroform has also been studied by: Regnaud, Villejean, Breteau and Woog, Baskerville, Clover, and others.

The first was that of Bigelow¹⁰ in 1898, who observed that a number of substances, such as benzyl alcohol, benzaldehyde, butyl alcohol, glycerol, mannite, cresols, aniline, et cetera, possessed the property of retarding the atmospheric oxidation of solutions of sodium sulfite. A considerable amount of research has been carried out on this subject.¹¹

Stannous chloride. Young¹² in 1901 observed a number of facts concerning the action of stannous chloride which were analogous to those observed by Bigelow with sodium sulfite.

Oxalic acid. It was observed by Jorissen and Reicher in 1903 that hydroquinone and resorcinol, among other substances, exerted a strong inhibitory action on the oxidation of solutions of oxalic acid by air.¹³

Alkaloids. Richard and Malmy in 1921 observed that sulfurous acid had a stabilizing influence on solutions of adrenaline.¹⁴ There is reason to believe, although the authors do not so state, that this is an anti-oxygenic action. The same may be said of the interesting observations of Bridel in 1923, on the preservation of solutions of various alkaloids by means of sodium bisulfite.

Many other substances, more or less clearly defined in their chemical composition, are also subject to oxidation in the air. In some instances their use is limited by this serious inconvenience. Various practices, which involve an anti-oxygenic action, have been employed to avoid these undesirable effects.

Paraffin. Siebeneck¹⁵ has recently observed that traces of sulfur prevented any oxidation of paraffin in free oxygen up to a temperature of 135°.

Fats and oils. Deschamps in 1843 showed that fresh lard containing gum benzoin or populin, did not become rancid as did pure

¹⁰ "Effect of Catalytic Action on the Velocity of Oxidation of Sodium Sulfite by Atmospheric Oxygen," *Z. phys. chem.*, **26**, 493 (1898).

¹¹ Young (1901), Titoff (1903), Lumiere and Seyewetz (1905), Dhar (1922).

¹² "Studies on Solutions of Stannous Salts. II. The Oxidation of Solutions of Stannous Chloride by means of Free Oxygen," S. W. Young, *J. Am. Chem. Soc.*, **23**, 119 (1901). Mittra and Dhar also worked on this subject (1922).

¹³ Handel Vlamsch Natuur- en Geneesk Congres, Ghent, Sept. 27, 1903.

¹⁴ *J. Pharm. Chim.* (7), **23**, 209 (1921).

¹⁵ *Petroleum*, **18**, 281 (1922).

lard.¹⁶ The cause of this preservation is attributed by the present authors to the anti-oxygenic action of phenolic substances present in benzoin (vanillin), or in the poplar buds (saligenin and derivatives).

Chevreur¹⁷ pointed out that he found oak wood to be a remarkable anti-drying agent for linseed oil; that poplar wood seemed to have this same property in a less degree; and pine wood in a slighter degree. It has been shown by the authors that phenols are excellent anti-drying agents for linseed oil, and that they act as anti-oxygens. We attribute the facts discovered by Chevreur to the presence of a certain quantity of tannin in the wood which he used, especially since it is well known that a considerable amount of tannin is present in oak.

Rubber. Several substances have been found to aid in the preservation of rubber. It is stated in a German patent¹⁸ that synthetic rubber is protected against auto-oxidation by the presence of phenolic substances.

Silk. Silk, as well as other animal fibres, is subject to more or less rapid alteration, especially when it has been impregnated with salts of tin. Various substances are claimed to act in a protective manner: thiocyanic acid, its salts, and other derivatives.¹⁹

Living tissues. It appears that Claude Bernard²⁰ was the first to note the inhibitory effect of small quantities of hydro-

¹⁶ J. Pharm. Chim. (3), 4, 201 (1843). According to Sisley, (1922), acetylthiourea also prevents fats and oils from becoming rancid.

¹⁷ Ann. chim. phys. (3), 47, 209 (1856).

¹⁸ Badische Aniline und Soda Fabrik, D. R. P. 330, 741 (1918). Other observations have also been made: Baeyer and Co., D. R. P. 366, 114 (1918); Helbronner, French pat. 509, 667 (1919), mentions an empirical procedure of vulcanization which depends as we shall show later, on an anti-oxygenic property.

¹⁹ Soc. Anon. cooperativa per la stagionatura e l'assaggio delle sete ed affini, French pat. 347, 689 (1904). The same problem was studied later by Herzog (1909), Otto Meister (1910), Berg and Imhoff (1910), Gianoli (1911), Sisley (1911), Korselli (1917).

²⁰ "Lecons sur les effets des substances toxiques et medicamenteuses," (1857). That the action of hydrocyanic acid is manifestly anti-oxygenic in nature, has been shown by other investigators, who did not work with such complex chemical materials as living tissues, but with pure chemical individuals (Abderhalden, Warburg, etc.).

cyanic acid upon oxidation by means of free oxygen in living organisms.

No doubt many other facts are scattered throughout the literature on this subject, and perhaps there are some which have escaped attention. There are a number of instances where the facts are difficult to understand, and cases where anti-oxygenic action does not appear sufficiently well defined have not been considered.²¹

It is evident that the problem of avoiding the undesirable activities of atmospheric oxygen, has led to many interesting observations. The publication by the present authors of numerous facts concerning anti-oxygenic catalysis, has drawn attention to the possibility of avoiding these oxidations and the resulting inconveniences. The publication of a theory as to their mode of action has served not only to coordinate the known facts and to guide the development of investigations, but has stimulated research upon the part of other scientists and in the industries, that has led to important discoveries. The present authors are collecting these experimental results as they are published, but will wait for some time before bringing them together into a systematic survey.

The wide extent of the field of anti-oxygenic action has been indicated by the variety of facts presented in the fore-going résumé. Our investigations in this field, which are summarized below, have indicated that a complete body of doctrine may be evolved about the phenomena of anti-oxygenic action. Instead of an unusual or rare occurrence, as one might be led to believe from the facts that have been presented thus far, we are dealing with an extremely general phenomenon that plays a rôle of great importance in natural processes. In fact, one might have been

²¹ Thus, certain materials which are contaminated with products of pyrogenation and are consequently liable to contain phenolic substances, are less susceptible to the action of air than the same substances in a high state of purity. This is the case when one compares certain salts of pyroligneous acid with the pure acetates. In the same way, disinfection by means of sulfur candles may often, in addition to the usual antiseptic effects, have an anti-oxygenic action about which little is known.

led long ago to suspect something of this nature, from a consideration of the simple fact that organic matter, endowed with a great reactivity toward oxygen, is capable of existing alongside this element in a free state in the atmosphere. Does it not seem probable that strong catalytic influences must be opposing these reactions, at least in a great number of cases, where until recently, we have been satisfied with the too vague notion of *passive resistance*?

II. OUR RESEARCHES

Origin of the study

In our first publication on the anti-oxygens,²² which appeared in 1922, we presented the fact that a wide variety of auto-oxidizable substances were protected against the action of oxygen by an entire class of chemical compounds; namely, substances having at least one free phenolic group. We announced at this time that the phenomenon was catalytic in nature, and that substances other than phenols might have the property of acting as anti-oxygens.

In a very fortuitous way, this subject presented itself in the study of a problem of the war. In order to make possible the use of acrolein, it was necessary to prevent the alteration of this extremely unstable substance. In spite of the investigations of a number of able scientists, the instability of acrolein remained as closely associated with it as its irritant properties. Furthermore, the product of its alteration, disacryl, is a stable resin from which it is impossible to regenerate acrolein. These facts proved to be serious difficulties in the use of acrolein; it was necessary to use the material immediately after preparation, and it could not be made in large quantities and stored.

After an empirical process of stabilization²³ had been found, we were successful in discovering by means of a systematic study, that phenols prevented the transformation of acrolein into disacryl. The protective action of certain phenols was very

²² "Sur l'autoxydation: les Antioxygènes." *Compt. rend.* 174, 258 (1922).

²³ Ch. Moureu and A. Lepape, *Compt. rend.* 169, 705 (1919).

powerful; for example, in the presence of one part of hydroquinone in ten thousand, acrolein remained absolutely transparent and unaltered, whereas a control sample soon became opaque and rapidly changed to an inert mass of disacryl.

The problem of the stabilization of acrolein was thereby completely solved and it was possible to manufacture this substance in large quantities in the French factories. In one factory it was possible to produce one thousand kilograms of acrolein per day, whereas the greatest quantity of acrolein which had been obtained before this work was that used by Emil Fischer in his celebrated synthetic work on the sugars. For this work Fischer used a quantity of two kilograms, which was considered at that time as an enormous amount of acrolein.

One might well ask, what relationship exists between the alteration and stabilization of acrolein and the problem of auto-oxidation? At the time of these investigations we did not suspect that our researches bordered on the field of auto-oxidation in its broadest conception. This is but another instance where a systematic scientific investigation has led into the most unexpected fields.

In attempting to elucidate this curious action of phenols we were led to the realization that auto-oxidation was the key to the mystery. It would be superfluous to mention the details and the difficulties encountered in the circuitous route that led to the idea of the following experiment,²⁴ apparently quite absurd, which was carried out in our laboratory on October 21st, 1917. In a barometer tube containing an atmosphere of oxygen over a mercury reservoir, was placed a sample of acrolein, which is known to absorb oxygen readily, and a trace of a substance of the type of pyrogallol, which is commonly used for the absorption of oxygen, was added with the hope that under these conditions oxygen would not be absorbed. The idea of this paradoxical experiment was the result of a careful analysis of many experimental observations; we were not surprised to note that the mercury level remained constant and consequently no oxygen was

²⁴ On this subject consult: "Les alterations de l'acroléin et les antioxygènes," Bull. soc. chim. (4), 31, 1152 (1922).

absorbed. A trace of a substance as easily oxidized as pyrogallol is thus capable of rendering oxygen inert toward another substance which is readily oxidizable in a pure state.

We trust that the description of the circumstances which were the origin of our researches on auto-oxidation will not be found out of place. This seems to us a typical example of the advantage of a thorough investigation of a phenomenon of which the nature is unknown.

Survey of the principal results

Preliminary experiments along various lines soon indicated that the phenomenon of anti-oxygenic action was extremely general in character. Although one might be led to believe the contrary from the small number of isolated and apparently unrelated experiments which were previously reported, anti-oxygenic action is widespread and is quite common. It is probable that under suitable conditions all oxidizable substances may act as anti-oxygens. Thus, in actual practice, perhaps an anti-oxygenic action is not exceptional, but rather the absence of all anti-oxygenic impurities is exceptional, in the system: auto-oxidizable substance plus oxygen.

Before taking up an investigation of the vast field of research which had thus been opened, we attempted to set up a theory which might serve as a guide to the immense number of experiments which would be necessary. From these theoretical considerations, which will be discussed in the second section, we undertook a systematic series of experiments with varied auto-oxidizable substances and catalytic agents.

We are now in the midst of the development of this problem which touches upon many of the different fields of chemistry, and our theory has thus been subjected to rigorous trial. By means of our theory, we have not only been able to explain every fact which we have encountered, but we have also been able to predict certain effects which would have been difficult to visualize without it. We shall consider this subject at length in the theoretical discussion, and it will suffice to point out here, three particularly striking results:

1. *Relationship of the two inverse catalyses.* The rational development of our theory led us to believe that a very close relationship existed between the two inverse catalyses of auto-oxidation, negative catalysis (anti-oxygenic action) and positive catalysis (pro-oxygenic action), and that there was probably a common underlying causation, such that a given catalyst might, according to the conditions, act either as an accelerator or an inhibitor of auto-oxidation. Our experimental results were in complete accord with this prediction.

The hydroiodide of methyl amine, for example, acts as an anti-oxygen toward benzaldehyde, but as a pro-oxygen toward styrol. Ethylxanthogenamide inhibits the auto-oxidation of an aqueous solution of sodium sulfite if the latter is slightly alkaline, but it behaves as an accelerator if the solution is slightly acid. It has been noted that substances which are the most active as anti-oxygens, are also the most active when they become pro-oxygens.

According to the particular case, a given catalyst may thus act either as an anti-oxygen or a pro-oxygen, and one cannot say that a given substance is an anti-oxygenic catalyst, or that another is a pro-oxygenic catalyst. To both of these, one must apply the generic term, auto-oxidative catalyst, since the sense of the catalysis, positive or negative, is dependent upon the conditions of the experiment and not upon the nature of the catalyst. The only property which is dependent upon the nature of the substance acting upon an oxidizable material, is that of being able to act as an auto-oxidative catalyst.

It is scarcely necessary to remark that the property of inversion of the sense of auto-oxidative catalysts has no connection with the well known reversibility of catalytic action. For example, hydrogen ions are known to catalyze the esterification of an alcohol with an acid (formation of an ester and water), as well as the hydrolysis of an ester by water (regeneration of the acid and alcohol), so that the final state of stable equilibrium, defined by the respective concentrations of the four constituents—alcohol, acid, ester, water—is the same whether it is attained from the system, alcohol plus acid, or the system, ester plus water, without

the intervention of a catalyst (although in reality a much longer time would be required). In auto-oxidative catalysis, on the contrary, there is not a state of equilibrium between the constituents and there can be no reversibility in the classical sense of the term. No catalyst has the power of regenerating benzaldehyde from the ultimate product of its auto-oxidation, benzoic acid. We shall return to this subject in the following section to discuss it from the standpoint of energy relationships.

2. *The catalytic properties of a substance are related to its susceptibility toward oxidation.* As we shall point out in the following section, our theory of auto-oxidative catalysis rests entirely upon the relationship between the susceptibility of a substance toward oxidation and its catalytic properties. We mention here a single striking example: phosphorus sesquisulfide (P_4S_3), which is used in the manufacture of matches on account of its great reactivity toward oxygen, acts as an effective anti-oxygen toward benzaldehyde at a dilution of one part per thousand.

3. *Generalization of anti-oxygenic and pro-oxygenic phenomena.* We have found more or less accentuated catalytic properties, under favorable circumstances, among several hundred compounds, all of which are susceptible to oxidation. We have succeeded in demonstrating by a direct method, that the catalytic activity was localized in that portion of the molecule which was oxidizable under the conditions of the experiment. All of our observations lead us to believe, as we might predict from the theory, that we could extend our list of catalysts almost indefinitely, from the most common chemicals to the rarest of them. In principle, any oxidizable substance whether its reactivity toward oxygen be great or slight, should be capable of acting as an auto-oxidative catalyst under the proper conditions. This idea confirms the statement made above; namely, that anti-oxygenic catalysis is a very general phenomenon, since we have everywhere matter which is susceptible to oxidation. We might even go further and state that an auto-oxidizable substance may act either as a positive or negative auto-oxidative catalyst toward itself.

Experimental Technic and Observations

Anti-oxygenic action. 1. Let us take a glass barometric tube, with the upper end bent in the shape of an inverted U and connected to a small bulb containing pure benzaldehyde, a substance which oxidizes rapidly in the air. Pure oxygen is introduced into the barometer tube until the mercury column within the tube is at the same level as that of the exterior. The mercury column within the tube soon starts to rise gradually. After several hours, when the height of the column indicates an internal pressure of 50–60 millimeters, the rise becomes slower and slower, and after twenty-four hours the internal pressure is still 20 to 30 millimeters. This phenomenon is due to the transformation of benzaldehyde, by the fixation of oxygen, into its peroxide and finally into benzoic acid.

If this experiment be repeated with benzaldehyde containing a small amount of hydroquinone (of the order of one part per thousand), the mercury does not rise and there is no absorption of oxygen. Thus, a trace of hydroquinone prevents the fixation of oxygen by benzaldehyde.

We have observed analogous results of approximately the same order of intensity, in replacing hydroquinone by other phenols, as well as a large number of other substances extremely varied in nature; on the other hand, these catalysts were found to exhibit a similar anti-oxygenic action toward a great variety of auto-oxidizable substances. Among the catalysts may be cited: catechol, pyrogallol, naphthols, tannins; iodine, inorganic iodides, ammonium iodide and substituted ammonium iodides, alkyl iodides, iodoform, carbon tetraiodide; sulfur, phosphorus sesquisulfide, inorganic sulfides, thioethers, mercaptans; aliphatic and aromatic amines, nitriles, amides, ureas, urethanes, substituted hydroxylamines, and dyes. Among the auto-oxidizable substances were the following: simple aliphatic aldehydes, such as acetaldehyde and heptaldehyde; substituted aliphatic aldehydes, such as acrolein and chloral; cyclic aldehydes, such as benzaldehyde, cinnamic aldehyde, and furfural; unsaturated hydrocarbons, such as styrol, phenylbromoethylene, diphenyl-

ethylene, spinacene, essence of turpentine; complex organic substances, such as caoutchouc, fats and oils; sodium sulfite, and other inorganic salts.²⁵ The anti-oxygenic activities were, of course, found to vary considerably according to the nature of the auto-oxidizable substance and the nature of the catalyst, as well as with the concentration of the latter.

In practice, a number of experiments was carried out in series. A number of modified barometric tubes were sealed, side by side, to a horizontal tube which was connected with a mercury reservoir. Special auxilliary connections were arranged in such a way that the series of bulbs and tubes could be evacuated to a very low pressure and filled with pure oxygen (fig. 1). The heights of the mercury columns were noted at regular intervals, varying from several minutes, or hours, to several days or weeks, according to the rate of absorption. In every set of tubes one

²⁵ Charles Moureu, Charles Dufraisse, and others: Sur l'autoxydation: les Antioxygènes, *Compt. rend.* 174, 258 (1922); II Le pouvoir antioxygène. Phénomènes divers se rapportant à l'action antioxygène, *Ibid.*, 175, 127 (1922); III Les altérations de l'acroléine et les antioxygènes, *Bull. soc. chim.* (4) 31, 1152 (1922); IV Autoxydation et Action Antioxygène, *Annales soc. espan. fis. quim.*, 20, 383 (1922); V Essai sur le mécanisme de l'action antioxygène, *Compt. rend.* 176, 624 (1923); VI Propriétés catalytiques de l'iode et de ces composés, *Ibid.*, 176, 797 (1923); VII Propriétés catalytiques de l'iode et de ces composés. Généralisation du phénomène, *Ibid.*, 178, 824 (1924); VIII (with Panier des Touches) Propriétés catalytiques des phénols iodes, *Ibid.*, 178, 1497 (1924); IX Propriétés catalytiques du soufre et de ces composés, *Ibid.*, 178, 1861 (1924); X (with M. Badoche) Propriétés catalytiques du soufre et de ces composés. Généralisation du phénomène, *Ibid.*, 179, 237 (1924); XI Activation et Désactivation de l'oxygène, *Rec. trav. chim.*, 43, 645 (1924); XII (with M. Badoche) Recherches sur la forme active autoxydable de l'acroléine, *Compt. rend.* 179, 1229 (1924); XIII Recherches sur la désactivation de l'acroléine par hydroquinone. Expériences à la lumière: l'action désactivante de l'hydroquinone ne peut suffire à expliquer son action anti-oxygène, *Bull. soc. chim.* (4) 35, 1564 (1924); XIV (with M. Badoche) Recherches sur l'activation de l'acroléine par la lumière, *Ibid.* (4) 35, 1572 (1924); XV (with M. Badoche) Recherches sur l'activation de l'acroléine par l'oxygène et par la lumière et sa désactivation par l'hydroquinone, du point de vue de sa condensation en disacryle, *Ibid.*, (4) 35, 1591 (1924); XVI (with P. Lotte) La propriété catalytique est localisée dans la partie oxydable de la molécule du catalyseur, *Compt. rend.*, 180, 993 (1925); XVIII (with M. Badoche) Actions catalytiques des composés azotés. Considérations générales, *Ibid.*, 182, 949 (1926); Reports of the Solvay International Council on Chemistry, April, 1925.

The accompanying curves (figs. 2 and 3) give an idea of the effects which were observed. It is worthy of note that one of the catalysts chosen as an illustration (fig. 3), namely, sulfur, is as powerful an anti-oxygen toward benzaldehyde as hydroquinone; considering the feeble reactivity of sulfur at ordinary temperatures, this pronounced activity seems quite remarkable.



A line graph showing the rate of reduction of various compounds by hydrogen gas over a 24-hour period. The vertical axis (y-axis) is labeled 'Mercury in millimeters' and ranges from 0 to 720 mm. The horizontal axis (x-axis) is labeled '24 hrs'. Several curves originate from the origin (0,0). The steepest curve is labeled 'Phosgene'. Below it is a curve labeled 'Benzaldehyde'. To the right of these is a curve labeled 'Hydroquinone'. Below the 'Benzaldehyde' curve is a curve labeled 'Zinc'. At the bottom of the graph, there are two more curves labeled 'Zinc' and 'Zinc', indicating much slower rates of reduction.

When the substance under investigation has a high vapor tension the general aspect of the curves is slightly different (fig. 4). With acrolein (b.p. 52°) the mercury rises in the control tube containing the pure substance, but in the tube containing the catalysts the mercury level falls until a certain level is reached, on account of the diffusion of acrolein vapor.

2. The above method often permitted us to distinguish quite sharply, differences between relatively slight variations in the concentrations of the anti-oxygen. In several instances, we were able to determine the sensitiveness of the anti-oxygenic activity, and it was found to vary considerably with the various catalysts.

The accompanying table (fig. 5) illustrates the effect of varying concentrations of hydroquinone upon the auto-oxidation of acrolein over a period of twenty-four hours. One will note that an appreciable effect is observed even in a concentration of one part per million.

If methylamine hydroiodide is used in place of hydroquinone, the effects are of the same order of magnitude (fig. 7).

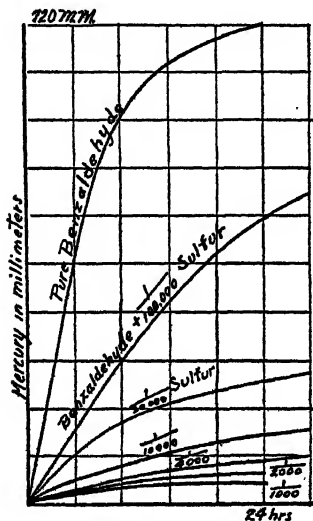


FIG. 3

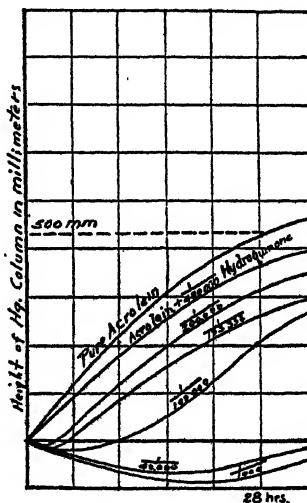


FIG. 4

FIGS. 3 AND 4. CURVES SHOWING RISE OF MERCURY DURING OXIDATION IN THE PRESENCE OF HYDROQUINONE

3. As we have just pointed out, the intensity of anti-oxygenic activity of a given catalyst is dependent upon the concentration. This leads to a consideration of the duration of this protective action, when the auto-oxidation appears to have been suppressed by a sufficiently high concentration of the anti-oxygen. If no special phenomenon occurred and the anti-oxygen remained intact, the protective effect should last indefinitely. In reality, it is found after a long period of time, that the anti-oxygen becomes altered by chemical reaction with the oxidizable substance, especially if fresh quantities of oxygen are frequently admitted

into the system; the quantity of anti-oxygen corresponding to this slow depletion is thus irreparably lost for catalysis. Nevertheless, our observations indicate that in a great many cases, such as acrolein containing one part of hydroquinone per thousand, the protective action may be considered to be practically indefinite.

A priori, it would appear quite simple to determine in a precise manner, whether an auto-oxidation which appears to be inhibited, is actually completely stopped, or only greatly retarded. It should be sufficient to prolong the experimental observations over an extended period of time; unfortunately, however, a parasitic reaction vitiates the results of such observations. If we continue to observe a series of tubes containing a substance under

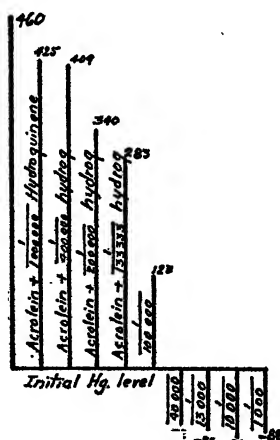


FIG. 5. TABLE SHOWING HEIGHT OF MERCURY AFTER TWENTY-FOUR HOURS

investigation with various catalysts, after a certain length of time (perhaps several days or weeks, or in some cases only after several months or years) we note an appreciable rise of the mercury column, indicating evidently a certain absorption of oxygen. On closer examination we find that a progressive condensation of the substance has occurred on the walls of the vessel and on the surface of the mercury, where it gradually collects. Obviously, it is the fraction of the material thus removed from the protective influence of the anti-oxygen which is responsible for the absorption

of the oxygen. This physical difficulty has limited the period of observation of our experiments and has prevented us from determining whether anti-oxygens actually stop the process of auto-oxidation or simply decrease the velocity of the reaction.

A simple experiment will serve as another illustration of the disturbing effect caused by vaporization and subsequent condensation of the auto-oxidizable substance. In our laboratory we have observed a corked bottle one-third filled with benzaldehyde, containing one part of hydroquinone per thousand, which has been allowed to stand undisturbed over a period of six years. Although the liquid appears unaltered except for a slight discoloration, the upper part of the bottle is coated with crystals of benzoic acid which evidently is formed by the oxidation of benzaldehyde which condenses upon the walls of the container (fig. 6).

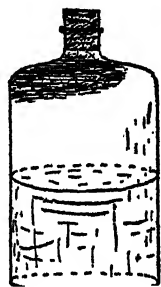


FIG. 6

A careful examination of the curves will show that the absorption of oxygen is never completely inhibited. Even under the most favorable circumstances a slight rise of the mercury column is observed if the experiment be sufficiently prolonged. We have now in progress a series of experiments in which the foregoing physical difficulty has been eliminated. The results of these observations will permit us to obtain precise data on the true velocity of oxidation in the presence of anti-oxygens.

Pro-oxygenic action. 1. Iodine and a number of iodine compounds act as positive catalysts toward the auto-oxidation of styrol. Similar behavior was observed toward linseed oil, as one might expect from the presence of double bonds in the latter, which make it analogous to styrol.

The green sulfide and the anhydrous pink sulfide of manganese accelerate considerably the auto-oxidation of benzaldehyde. Cobalt sulfide is also an accelerator but is less active than the sulfides of manganese.²⁶ Other positive catalysts for the auto-

²⁶ Conversely, iron sulfide and nickel sulfide behave as anti-oxygens. It appears that this activity is not a specific property of the metallic ion, which may pro-

oxidation of benzaldehyde are carbon disulfide, sodium sulfide, and diethyl thiocarbonate.

Among certain interesting facts which have resulted from our observations may be mentioned the following:

Ethyl sulfide acts as a pro-oxygen toward acrolein, although ethyl mercaptan is a strong anti-oxygen toward this substance.

Phosphorus sesquisulfide, thiophenol, ethyl mercaptan, antimony sulfide, act as positive catalysts toward styrol.

Thiophenol and the green sulfide of manganese are accelerators toward essence of turpentine.

Phosphorus sesquisulfide and the green sulfide of manganese are accelerators toward linseed oil.

2. In general, the activity of pro-oxygenic catalysts was found to be relatively slight, although we have encountered instances where the speed of auto-oxidation was increased one hundred fold. Usually the coefficient of acceleration falls between 2 and 20. We have not ordinarily taken into account any cases where the coefficient was less than 2.

One must not confuse the phenomena of pro-oxygenic activity with certain others of a somewhat similar nature which are distinctly different. Numerous substances which are practically inactive toward free oxygen, are readily oxidized in the presence of an appropriate catalyst. The latter first auto-oxidizes, then gives up to the substance the oxygen thus acquired, and is itself regenerated in its original unoxidized state; the cycle then recommences and goes on indefinitely.

A clear cut example of this type of oxidative catalysis (involving preliminary auto-oxidation of the catalyst) is furnished by the oxidation of glucose by free oxygen in the presence of cerium salts, discovered by Job.²⁷ A solution of glucose containing a trace of a cerous salt, oxidizes in the air according to the mechanism just described, and the cerous salt acts in this instance as an *auto-oxidizing catalyst*.

duce either one of the two opposite effects. In fact, we have observed that the chlorides of nickel and manganese are accelerators toward benzaldehyde, but the iodides are inhibitors. It can also be seen that manganese does not always have pro-oxygenic activity as one might be led to believe a priori.

²⁷ Ann. chim. phys. (7) 20, 205 (1900).

Many other examples involving auto-oxidizing catalysts are known, such as the oxidation of hydroquinone and pyrogallol under the influence of traces of manganous salts, or under the influence of laccase, a soluble enzyme containing manganese.²⁸

Inversion of the catalysis. 1. On account of the close relationship which exists between the two opposite types of catalytic action, an inversion of the sense of catalysis frequently occurs. Our investigations on this subject have resulted in a number of sharply defined observations. In addition to those which have already been mentioned are the following:

Iodine, which is a powerful anti-oxygen toward benzaldehyde, is a pro-oxygen toward styrol.

Iodoform, which is a pro-oxygen toward styrol, is an anti-oxygen toward furfural.

Phosphorus sesquisulfide, which is an anti-oxygen toward benzaldehyde, is a pro-oxygen toward linseed oil.

Thiophenol, which is an anti-oxygen toward benzaldehyde, is a pro-oxygen toward essence of turpentine.

Sulfur and diethylene disulfide, which inhibit the auto-oxidation of sodium sulfite in alkaline solution, behave as accelerators if the solution is slightly acid.

2. Ordinarily, in these inversions, the most powerful anti-oxygens become also the most active pro-oxygens. For example, methylamine hydroiodide is an extremely active anti-oxygen toward acrolein; conversely, it is a highly active catalyst as a pro-oxygen toward styrol.

3. In certain observations of the action of a catalyst upon the auto-oxidation of a given substance, it was noted that the sense of the catalysis changed during the course of the experiment.

The behavior of iodine and iodine compounds toward acrolein furnished a number of examples of this inversion. The catalysis, at first negative, changed to positive after a certain length of time, which varied from several minutes or hours, to days or weeks, depending upon the particular catalyst and the concentration. This occurrence was observed in the following instances;

²⁸ Bertrand, *Ann. chim. phys.* (7) 12, 115 (1897).

methylene iodide, one per cent (negative catalysis during 15 minutes); iodoform, one per cent (20 hours); carbon tetraiodide, one per cent (several days); ethylene iodide, one per thousand (8 hours); acetylene di-iodide, one per thousand (18 hours); iodol, one per thousand (90 hours); benzyl iodide (70 hours).

The accompanying graph (fig. 7) shows the remarkable results obtained with methylamine hydroiodide in high dilutions. There was observed at first an intense anti-oxygenic activity, then oxida-

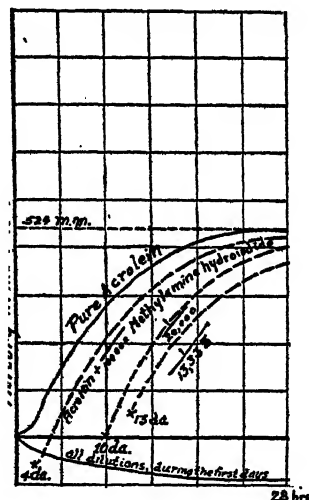


FIG. 7. CURVES SHOWING RISE OF MERCURY DURING OXIDATION IN THE PRESENCE OF AN HYDRIODIDE

The dotted curves have been brought toward the y -axis by parallel transposition.

tion started suddenly at a rapid rate which was about the same as that of the control tube of pure acrolein. In this case there was no well defined pro-oxygenic action, since the oxidation was not markedly accelerated, probably on account of the very great dilution of the catalyst. The positive activity being slight, did not appear distinctly in such very low concentrations, but the inversion was too distinct to leave any doubt.

Among other anomalous results which were noted are the following:

Phosphorus sesquisulfide acts toward turpentine, first as an accelerator, then as an anti-oxygen. Thiophenol acts in a similar manner toward linseed oil. Conversely, thiolactic acid and thio-glycolic acid exert first, an anti-oxygenic effect toward acrolein, followed by a pro-oxygenic activity.

These are but a few of the numerous examples of this type.

Secondary phenomena of auto-oxidation and anti-oxygenic action. Auto-oxidation is very often accompanied by secondary reactions such as molecular condensation, which is manifested by resinification, or the development of colorations, precipitates, or rancidity. When an anti-oxygen prevented the fixation of oxygen, we observed a simultaneous inhibition of these secondary reactions. Thus, furfural, which usually becomes rapidly discolored, remains almost colorless; acrolein does not become turbid from the precipitation of disacryl; styrol remains fluid and mobile, instead of being resinified with the formation of metastyrol; linseed oil, upon exposure to the air in thin films, remains fluid after three years; butter, and other fats and oils, do not, in general, turn rancid.

It appears that the immediate agency which produces these secondary phenomena, is not free oxygen itself, as one might suppose, but is combined oxygen from a peroxide which is first formed from the auto-oxidizable substance. This has been rigorously demonstrated in the case of acrolein in the following way.

If acrolein which has been exposed to the air, is freed from dissolved gases by thorough evacuation and is then preserved in a high vacuum, condensation and formation of disacryl proceed in the same manner as in the presence of air. Therefore, free gaseous oxygen is not responsible for this alteration, but contact with air has sufficed to inoculate acrolein with a mortal poison. On the other hand, if we allow acrolein containing a small quantity of an anti-oxygen to stand in contact with free oxygen, there is no condensation; consequently, it is not free oxygen which causes the alteration, but combined oxygen. We conclude from the above facts, that the catalytic agent which effects the conversion of acrolein into an insoluble resin is a peroxide.

Auto-oxidation of vapors. It would be natural to expect that the vapor of an auto-oxidizable substance, such as benzaldehyde, when mixed with an excess of gaseous oxygen, would be oxidized rapidly. If one were dealing with a system in which an excess of auto-oxidizable liquid were present, so that the depletion of vapor could be replenished as rapidly as oxidation occurred, one might think that the absorption of oxygen would proceed with greatest intensity in the vapor phase.

If we consider our experimental technic, in which the essential instrument is a barometer tube, and we examine the case of a catalyst which completely inhibits oxidation, one conclusion appears to be inevitable: the vapors of an auto-oxidizable substance do not undergo oxidation. In fact, with our experimental procedure, it would have been impossible to observe anti-oxygenic action,—at least in those most common cases where the anti-oxygens under investigation did not have an appreciable vapor pressure, and could not exert an anti-oxygenic influence in the gaseous phase. If there had been an appreciable oxidation in the gaseous phase we would have been unable to note a diminution in the rate of absorption of oxygen.

Our experiments have revealed very definite anti-oxygenic action, and at the same time, have established, not only that the vapor of an auto-oxidizable substance does not oxidize rapidly, but apparently that oxidation does not occur at all in the vapor phase (under the conditions of our experiments).²⁹ This is a rather important and altogether unexpected result.

Repercussions in various fields. Anti-oxygenic and pro-oxygenic catalysis may play an important rôle in the chemical processes involved in a multitude of natural phenomena, both in inanimate matter and in living organisms. We shall enumerate and discuss certain special cases.

1. *Contact catalysis—the so-called poisoning of catalysts.* We shall be concerned here with contact catalysis only inasmuch as it involves phenomena of auto-oxidation. Outside of living organisms, with which we shall be concerned later, this is true

²⁹ Experiments relative to this point are now in progress.

in a certain number of cases, particularly those of catalysis by free metals. In these cases, we suppose that the catalyst auto-oxidizes to give an unstable oxide, which in turn gives up its oxygen and regenerates the metal. It has long been known that platinum in a finely divided state (platinum sponge, platinum black, platinized asbestos, and colloidal platinum), is an active catalyst for a variety of oxidations. Other metals of the platinum group, particularly palladium, may behave in a similar manner, and the same is true of gold, silver, and copper.

We can consider in the same light other catalytic agents which undergo preliminary auto-oxidation. For example, recent studies have shown that a large number of organic substances may be completely oxidized at ordinary temperatures in the presence of charcoal; mixtures of metallic oxides, such as "hopcalite," which has been extensively studied in America, have the property of oxidizing carbon monoxide at room temperature.

It is well known that such catalytic activity is often inhibited by traces of certain substances which behave as catalyst poisons. Thus, small quantities of certain gases or vapors (ethylene, carbon monoxide, hydrogen sulfide, and others), prevent platinum sponge from effecting a violent combination of hydrogen and oxygen at room temperature. We believe that these inhibitory substances exert the same anti-oxygenic influence upon the transitory oxidation of platinum, that hydroquinone exerts toward benzaldehyde, and we would apply the general mechanism of anti-oxygenic action which will be discussed later, to explain this so-called "poisoning" of oxidizing catalysts.³⁰ We believe that our interpretation may also be applied to a number of other instances of the poisoning of oxidizing catalysts.

2. "*Anti-knock*" agents. The recent discoveries of the American chemist, Midgley, have shown that small amounts of various substances have a remarkable effect in preventing knocking in motors. We believe that here also, one is dealing with an anti-oxygenic action which is quite comparable with those that have already been described.^{30a} We might add in this connection, that

³⁰ Moureu and Dufraisse, *J. Chem. Soc.*, 127, 1 (1925).

^{30a} Cong. Soc. Chim. Ind., Paris; October, 1925.

tetraethyl lead, which is a powerful anti-knock agent, is also capable of acting as an anti-oxygen toward benzaldehyde at room temperature. Tetraethyl lead acts as a moderator to the action of oxygen at a high temperature upon the explosive gas mixtures of motors, and it exerts the same moderating influence on auto-oxidation under the usual conditions of our experiments. Its mode of action would seem to be the same in both cases.

3. *Biological considerations.* On account of the fundamental interest of auto-oxidation in vital phenomena, from the very first we were able to perceive numerous applications of the facts which we had discovered to several general conceptions of biology.

Phenols. Phenols are present in many living organisms, but from this standpoint there are two distinct categories of organisms; those in which phenols are quite common and wide spread, and those in which phenols are rare. The first group is made up of plants and trees, where one encounters an abundance of the most varied phenolic compounds; monohydric and polyhydric phenols and their derivatives, among which the tannins merit special notice, on account of their general distribution and occurrence in large quantity. The second group is made up of animals; among these, very few phenols occur, and always in very small amounts. Plants are precisely the organisms of slowest metabolism, and those in which the phenomena of auto-oxidation do not have the same intensity as in animals, and it is but natural to suppose that in animals, the phenols act as protective agents against a too vigorous oxidizing action. One cannot help but remark the fact that the tannins, which are very active phenolic substances, are present in greatest amounts in those parts of the plant in which metabolism is at the lowest ebb.

Among the most interesting experiments which suggested themselves for consideration, was the auto-oxidation of hemoglobin. We have carried out a number of experiments in this field, with hemolysed blood, and with the liquid resulting from the hemolysis of washed red corpuscles, and with solutions of hemoglobin which had been purified by crystallization. Our results were negative with the various phenols investigated, namely, phenol, naphthols,

catechol, guaiacol, resorcinol, hydroquinone, pyrogallol, gallic acid. Hemoglobin which had been reduced by evacuation at 40° was found to oxidize as really in the presence of phenols as in their absence. We observed what appeared to be a mutual destruction of both the phenol and hemoglobin.

One must not necessarily conclude from these results that phenolic substances are without action on the auto-oxidation of hemoglobin. The speed of auto-oxidation of hemoglobin is enormous, perhaps comparable with the neutralization of a strong acid with a strong base; it is therefore quite possible that the antagonistic action of the phenols was too weak to be noticeable under the conditions of our experiments.

Nevertheless, it is probable that phenolic substances play some part in a stage of the oxidative mechanism of higher animals. This contention is supported by the toxicity of the phenols; those which are the most active anti-oxygens are the most toxic, and the symptoms of poisoning are similar to those of asphyxia.

It seems likely that the antiseptic properties of phenols may be due to their anti-oxygenic activity, which perhaps affects microorganisms by interfering with their oxidative processes.

When one considers the extremely minute quantities of an anti-oxygen which are capable of producing very definite effects, one can scarcely avoid comparing their activity to that of toxins and venoms. A certain number of the latter cause death by asphyxiation, and this leads to the idea that they may act as anti-oxygens. This viewpoint would be supported by the fact that quinone has been encountered in certain venoms.

Finally, we cannot omit to mention certain consequences which may result from the application of this new notion of anti-oxygens to pharmacology. It is particularly remarkable that phenols are antipyretics, no doubt, on account of the fact that they attenuate the intensity of oxidative metabolism. This is perhaps the key to the physiological action of antipyretics in general. In fact, it happens that antipyretics used in therapy are aromatic substances, and it has been observed that aromatic compounds may be oxidized in the body to give phenolic substances. It is evident that a knowledge of the anti-oxygenic properties

of phenols may be of such a nature as to modify the interpretation of certain therapeutic effects which are observed to follow their administration. From this conception one cannot help but note the favorable results often obtained by the use of phenolic substances (creosote, guaiacol and its derivatives, and others) in the treatment of a malady which closely affects the oxidation processes of the organism, namely, pulmonary tuberculosis. It would fall into the domain of workers in this field to determine whether or not these substances act in moderating respiratory hyperactivity, as well as destroying microorganisms.

Iodine and its derivatives. It is well known that iodine is widely diffused throughout the mineral kingdom. There are considerable amounts of iodides in sea water, in which we find innumerable living organisms which accumulate this metalloid in their tissues. We also find iodine in all forms of life, plant or animal, and it is ordinarily considered as one of the elements which are necessary for life (A. Gautier, Baumann, Gley, and others).

From our experiments one might suppose that iodine acts in a number of cases as a regulative agency for the phenomena of oxidation. Small quantities of energy would suffice to change iodine from the state of a negative catalyst to that of a positive catalyst, and vice versa, according to the needs of the organism.

Although the higher animals live in an environment very low in iodine, they concentrate this element in a special organ, the thyroid gland, to which we attribute precisely the rôle of regulating oxidative metabolism.

Hence it was natural to extend our experiments to extracts of the thyroid gland. Our results were quite well defined, but it is difficult to interpret them correctly, on account of the complexity of the material which we used. We hope to be able to investigate the behavior of thyroxin, the active principle isolated by Kendall, but we have not yet been able to obtain a sample of this substance.

Sulfur and its derivatives. Sulfur is present in many substances which occur in living organisms and is found in one of the most important groups of organic compounds of natural origin, the proteins.

The recent work of Hopkins³¹ and his collaborators has drawn attention to the presence of glutathione in practically all animal tissues. According to Hopkins, glutathione³² can exist in two forms, an oxidized form $R-S-S-R$ and a reduced form $R-S-H$, which are readily interconvertible. He states that this substance acts as an oxidizing catalyst, but differs from hemoglobin, which is an oxygen carrier, in that it is a hydrogen carrier. The latter transports hydrogen from an oxidizable substance to free oxygen and the result amounts to an oxidation of the oxidizable substance.

When an equilibrium between a substance X and its oxidation product Y , occurs among the phenomena of a catalytic oxidation, we have found that it is not necessarily this reversible transformation which is the basis of the catalytic activity. We have demonstrated that X and Y each have a specific catalytic influence. In the case of thiophenol, a catalyst which we have observed to be converted into diphenyl disulfide during the course of certain catalytic oxidations, the passage from $R-S-H$ to $R-S-S-R$ and vice versa, is in reality an accessory phenomenon of the catalysis, contrary to the statement of certain authors. Catalytic activity is produced according to a mechanism (which we shall discuss later) involving much more rapid reactions, which consequently result in much more intense catalytic effects.

Nitrogen compounds. From a biological standpoint nitrogen compounds are of much greater importance than any other substances, either organic or inorganic, which have been mentioned thus far. Nitrogen in various forms is an essential constituent of all living tissues and is present in the principal compounds

³¹ "The Mechanism of Oxidation in Living Organisms"—Lecture before the International Conference on Pure and Applied Chemistry; Cambridge, 1923, published in *Bull. soc. chim. biol.*, 5, 761 (1923); compare also Hopkins, *Biochem. J.*, 15, 286 (1921), Hopkins and Dixon, *J. Biol. Chem.*, 54, 529 (1922); Hopkins, *Biochem. J.*, 19, 787 (1925).

³² Glutathione is a dipeptide resulting from the union of cystine,
 $CO_2H-CHNH_2-CH_2-S-S-CH_2-CHNH_2-CO_2H$, or cysteine, $CO_2H-CHNH_2-CH_2-SH$
 with glutamic acid, $CO_2H-CH_2-CH_2-CHNH_2-CO_2H$.

which are elaborated by the organism. It is of fundamental importance in the movement of oxygen in higher animals. On account of the great complexity of many of these compounds one would scarcely expect to find their activity to be simple in nature. Studies which are now in progress may lead us to certain generalizations on this subject.

Practical applications. We have pointed out the desirability of being able, at will, to accelerate or diminish the reactivity of oxygen. Our experiments lead to hope that this problem may be solved easily. In our own experience we have been able to inhibit a number of undesirable oxidative effects and thus avoid their harmful consequences. We have been able to preserve many alterable compounds, such as unsaturated hydrocarbons, aldehydes, delicate perfumes, caoutchouc, fats and oils, et cetera.

We employ anti-oxygens currently in our laboratory for the stabilization of various auto-oxidizable substances. A striking example of the practical service which anti-oxygens render almost every day, is the case of furyl ethylene, $C_4H_3O-CH=CH_2$. This extremely sensitive substance was the starting point in a study which was undertaken in our laboratory with the collaboration of an American student. If we had not been able to stabilize furyl ethylene and to assure ourselves of a sufficient supply of this material, it would have been necessary to renounce the work and we would have been unable to achieve the goal, which was furyl acetylene, $C_4H_3O-C\equiv CH$.

The advantages of the use of anti-oxygens are not limited to the preservation of sensitive substances against oxidation. Traces of peroxides are often known to produce disastrous catalytic effects upon the yields in certain chemical operations, particularly on account of many condensation reactions which they bring about. It is therefore advantageous to utilize anti-oxygens, even with substances that are comparatively unreactive toward oxygen, when it is necessary to allow an auto-oxidizable substance to come into contact with air. It has been found that acrolein gives much better yields in a Grignard reaction if it has been preserved against auto-oxidation by an anti-oxygen, rather than

simply stored out of contact with air.³³ An analogous result was obtained in the transformation of essence of turpentine into camphor.

Scientists in various parts of the world have honored our work by the utilization of anti-oxygens in their researches. Many were kind enough to write and inform us of the beneficial results which were obtained. Nevertheless, in the utilization of these catalysts one must not lose sight of the close relationship which exists between the two opposite senses of the catalysis: according to the conditions and to the influences of minute quantities of reagents, one may produce a pro-oxygenic instead of an anti-oxygenic catalysis. A catastrophe is thus brought about in the attempt to avoid it. In this connection one may recall, with a slight deformation of its meaning, an old saying: "The Tarpeian rock is near the Capitol."

2. THEORIES OF ANTI-OXYGENIC ACTION

Let us consider the system composed of an auto-oxidizable substance A, and oxygen O_2 . The stable state of this system corresponds with the formation of a stable oxide, AO_2 , since the inverse reaction $AO_2 \rightarrow A + O_2$, is not possible unless energy is furnished to the system. Let us add a catalyst B to the system—if we observe a diminution in the velocity of formation of AO_2 , we state that this activity which we call anti-oxygenic action, is a negative catalysis.

We have indicated in the preceding chapter, that the two modes of catalysis (positive and negative) mentioned here, are not to be confused with catalysis in the two opposite senses produced by the same catalyst in the well-known phenomenon of the inversion of catalytic action. In fact, if one considers this inversion one realizes that the action of the catalyst is always positive from the standpoint of the energy relationships—both when it accelerates the direct and the reverse reaction—since in each case it tends to bring the system into a state of stable equilibrium.

³³ The yield of vinylmethylcarbinol was increased from 25 per cent to 52 per cent by the use of acrolein containing one part of hydroquinone per thousand; Daudrenghein, Bull. soc. chim. Belg. 31, 160 (1922).

In the case of auto-oxidation the facts are not apparently so simple from the energetic point of view. Pro-oxygenic activity is undoubtedly a positive catalysis, since it favors the displacement of the system $A + O_2$ toward its stable state of equilibrium, corresponding to the stable oxide AO_2 . But what is anti-oxygenic activity? We have used in this connection the term *negative catalysis*, which is not particularly appropriate. In reality the term negative catalysis, which we did not create, does not appear especially fortunate. Although it expresses very well the superficial appearance of the phenomenon, that is, the production of an effect opposite to that produced by positive catalysis, it presents the serious inconvenience of lending itself to an inexact interpretation. It seems to imply the idea of a catalysis in the wrong direction; a catalysis which would give to the reaction an impulse in the opposite direction to that given by positive catalysis. In the case of a movable object, if one designates displacement in a certain sense positive, the corresponding velocity is positive, while a velocity corresponding to displacement in the opposite sense is negative.

It is evident that here an analogous point of view would be grossly erroneous. No catalyst is able, without the addition of external energy, to reverse the course of spontaneous chemical reactions which are accelerated by positive catalysts, for it is impossible to raise the potential of a system without the expenditure of energy, and the laws of thermodynamics require that when a phenomenon takes place spontaneously (that is, without the application of external energy to the system) it always takes place with liberation of energy. A diminution of potential, negative catalysis in the literal sense of that expression, is therefore impossible. For instance, no catalyst is able to regenerate from benzoic acid the initial mixture of benzaldehyde and oxygen. This would be in the strict sense of the word a true negative catalysis.

It follows that if anti-oxygenic activity is really a catalysis, as we have already called it by anticipation, it can be no other than a positive catalysis; that is, a catalysis impelling the system toward a state of equilibrium, a phenomenon permitting a lowering of potential.

In the case of reversible reactions, one can easily conceive that a catalyst might be capable of producing two inverse chemical reactions, depending upon whether it impels the reaction in one direction or in the reverse direction. Here, on the contrary, in the case of anti-oxygenic and pro-oxygenic activities we do not have an analogy, since we are dealing not with a reversible reaction but with the irreversible change, $A + O_2 \rightarrow AO_2$. We are therefore confronted with the problem of finding a mechanism for anti-oxygenic catalysis such that we shall deal with an equilibrium which is impelled in a sense opposite to that of pro-oxygenic catalysis, the only one of which we are able to perceive the result. In fact, if one considers the case of a stone on the side of a hill, one can compare positive catalysis to an agency which facilitates its sliding downward, but it is difficult to imagine a catalysis, also positive (corresponding to anti-oxygenic action) which would facilitate a movement that would result in maintaining the stone in its original position. That is, the mode of action must be such that as a result of the movement the stone returns to its original position. This problem of paradoxes is capable of solution, and we believe that it can easily be solved by the present resources of physical chemistry.

Before examining the possible interpretations, we shall consider first an hypothesis for the mechanism of anti-oxygenic action which does not involve a catalysis.

I. NON-CATALYTIC THEORY OF ANTI-OXYGENIC ACTIVITY

Does the action of anti-oxygens consist in a neutralization of the action of positive catalysts?

Various observations have led to the supposition that the process of auto-oxidation requires the aid of a positive catalyst. Such substances would always be present in a system whether or not one succeeds in demonstrating their precise rôle. The action of the walls of the vessels, minute amounts of impurities, and traces of moisture, appears to be essential in many reactions. It has, for example, been established that oxidations are difficult to

carry out with thoroughly dried oxygen;³⁴ that it is impossible to bring about the detonation of absolutely dry mixtures of carbon monoxide and oxygen, that a carbon monoxide flame is extinguished by absolutely dry air;³⁵ and that carbon and oxygen rigorously dried do not combine at 1000°.³⁶

Theory of Titoff. If, in certain cases, the presence of traces of appropriate impurities is necessary and sufficient to permit auto-oxidation, it should be comparatively simple, by the addition of small amounts of impurities to extinguish their effects. It was thus that Titoff³⁷ explained the hindering effect caused by traces of benzyl alcohol, benzaldehyde, mannite, glycerol, and various phenols, in the auto-oxidation of dilute solutions of sodium sulfite; he showed that copper is an extremely active positive catalyst for this reaction, and he came to the conclusion that the rôle of a negative catalyst is simply to paralyze (by complex formation) the action of a positive catalyst.

In a like manner, Warburg³⁸ concluded that iron in many biological and similar processes is neutralized by various substances, especially by hydrocyanic acid.

Although such explanations may appear sufficient in certain isolated cases, they are in general inadequate. If one remembers, for example, that traces of hydroquinone protect a great number of diverse substances against the action of oxygen, it is difficult to suppose that this substance really has the power in each case, to neutralize the positive catalyst.

In the first place, it is quite certain that in general, the positive catalyst which is neutralized cannot be water, as in certain examples cited above, since anti-oxygens are not dehydrating agents, and since moreover, they may act in aqueous solution.

In the second place, if hydroquinone owes its activity to a neutralizing action upon a positive catalyst, one would be forced to suppose that the most diverse substances susceptible to auto-

³⁴ Dixon, Trans. Roy. Soc. London, **37**, 56 (1884).

³⁵ Traube, Ber. **18**, 1890 (1885).

³⁶ Baker, J. Chem. Soc., **47**, 349 (1885); Proc. **1**, 37 (1885).

³⁷ Titoff, Zeit. phys. chem., **45**, 641 (1903).

³⁸ Warburg, Ber. **58**, 1001 (1925).

oxidation, owe this susceptibility to the presence of the same impurity, or at least to impurities belonging to the same category; and this appears very improbable. On the other hand, as we have shown (we shall return to this in more detail later), first, a common property of all anti-oxygens, and one which appears an essential condition for anti-oxidative activity, is oxidizability; second, according to the conditions, the same catalyst may act either to diminish or accelerate auto-oxidation, i.e., either as an anti-oxygen or as a pro-oxygen. These are two remarkable properties of anti-oxygens and they are very difficult to account for by means of the above hypothesis.

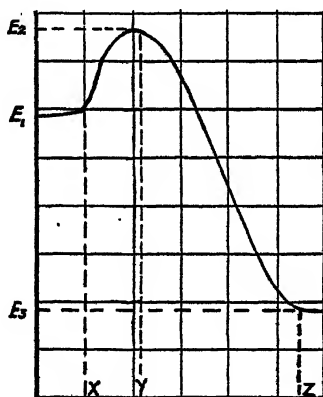


FIG. 8

II. THEORIES INVOLVING A CATALYTIC MECHANISM

If one is to conceive of anti-oxygenic action as a catalysis, the latter must necessarily be positive, and it is essential to concede that the phenomenon of auto-oxidation of a substance A, does not consist in a continual drop of potential of the system $A + O_2$ until oxidation is complete. One does not escape the fatality that the process is obliged to imply an increase of potential at a certain moment. It is at this phase that an anti-oxygen will oppose the ensemble of auto-oxidation processes by a positive catalytic action, that is by favoring a lowering of potential.

The accompanying curve (fig. 8) is a graphical representation,

in which time is the abscissa, and the ordinate is the potential energy of the mixture of the two molecules A and O_2 which are to react with each other. It is in the portion of the curve projected in XY that anti-oxygenic action would occur.

This conception was not invented by us for the needs of the moment. It is a relatively old notion of the great Swedish theorist Arrhenius, which is coming to play an increasingly important part from day to day in our modern physical theories. Due to its basis of solid experimental facts, none could be better suited to the interpretation of our results.

It is well known that physical and chemical observations have led to the inference that in a fluid (liquid or gas) made up of a definite chemical species, all of the molecules are not in the same state. From the standpoint of energetics the states of the individual molecules are distributed around a mean state and the proportion of molecules in a given state varies inversely with the difference from the mean state. Molecules removed from the mean state are called *activated*. In most chemical reactions all of the molecules present cannot interact simultaneously, and at a given moment only a small portion of the entire number can enter into reaction. The velocity of a reaction is regulated precisely by the proportion of active molecules, and by the speed with which they are formed in the ensemble of the mass. Due to developments of this theory by Berthoud, Marcelin, Lewis, Perrin, Langevin, and others, one may calculate the excess of energy which must be acquired by the molecules in a mean state to become activated for a given reaction. This minimum additional energy requirement is called the *critical increment of energy*.

The especially favorable condition of the active molecules is acquired at the expense of the average energy, in accordance with statistical laws.³⁹ Although the curves which we have given above may appear somewhat fantastic at first, they are readily explained on this basis. The average molecules are found at the level E_1 ; those which react must pass through the level E_2 before descending to the level E_3 , which represents the stable state of

³⁹ Moureu and Dufraisse, Reports of the Solvay International Council on Chemistry, Brussels, April (1925), p. 529ff.

auto-oxidation. At the moment of the obligatory passage through the level E_2 , two catalyses are possible, both positive of which the effects are diametrically opposite: one, pro-oxygenic action favoring movement to E_2 ; the other, anti-oxygenic action favoring return to E_1 . Thus, it is evident that if one succeeded in causing a constant retrogression of the molecules at the level E_2 to the level E_1 , the entire phenomenon of auto-oxidation would be suppressed.

This representation of the process of anti-oxygenic action readily explains one of the most striking characteristics of the phenomenon, namely, that a very small number of molecules of an anti-oxygen can prevent oxidation of an enormous number of molecules of an auto-oxidizable substance in contact with oxygen. There is something truly disconcerting about this fact; for example, one molecule of hydroquinone suffices to prevent the oxidation of forty thousand molecules of acrolein, and considerable effects may be observed at dilutions much greater than this. Such a disproportion between the number of molecules which tend to be oxidized and the number of antagonistic molecules leads to the conclusion that the former must pass through some critical transitory stage where they are particularly vulnerable, and that it is only in such a transitory stage that the opposing molecules are present in sufficient number.

Direct inactivation

Do anti-oxygens act through a process of direct inactivation of the molecules of either the auto-oxidizable substance, or of oxygen?

It is evident from the foregoing paragraphs that if one succeeded through any agency in diminishing the concentration of active molecules of a chemical species, one would diminish the velocity of reaction in the same measure. It would then be rational, a priori, to think of a simple mechanism in which the anti-oxygen, which we shall designate as B, would act in catalyzing the return of active molecules, either of the auto-oxidizable substance A, or of oxygen O_2 , to the mean energy level of the ensemble. From the outset we have been occupied with this

suggestion which has been put forward and supported by various scientists. In particular, Professor Hugh S. Taylor⁴⁰ in a paper on this subject, has made certain very suggestive calculations based upon our experimental results, and has presented a series of experiments in support of his views.

We have shown with benzaldehyde, for example, that a proportion of one part of hydroquinone per thousand produces very marked anti-oxygenic effects, so that at this very low concentration, benzaldehyde is practically stable in the air. Taylor points out that if, instead of reasoning from the total number of molecules of benzaldehyde, one consider only the molecules which are ready to react (i.e., the number of active molecules, which he determined by the speed of oxidation) hydroquinone is not only present in the liquid in stoichiometric superiority, but is present in an overwhelming proportion when compared with the number of active molecules of benzaldehyde. It would suffice, therefore, that the molecules of hydroquinone enter into a transitory combination with active molecules of benzaldehyde, and that this combination dissociate later with liberation of a molecule of benzaldehyde in an inactive state.

In order to support this theory with experimental evidence, Taylor investigated the tendency of benzaldehyde to form molecular complexes with various substances which we had found to act as anti-oxygens toward this aldehyde, and he sought the property of anti-oxygenic activity among substances which were known to form molecular complexes with benzaldehyde. Along with an undeniable measure of success, he encountered certain practical difficulties.

Notwithstanding, this theory is very interesting, and we have already refuted certain criticisms which have been advanced.⁴¹ In our opinion it seems necessary to supplement this theory by the following observations:

Selective attraction. We believe that the difficulties encountered by Taylor are centered in his idea of the tendency toward forma-

⁴⁰ J. phys. chem., 27, 322 (1923).

⁴¹ Moureu and Dufraisse, Reports of the Solvay International Council on Chemistry, Brussels, April (1925) p. 554.

tion of molecular complexes. As he justly stated, the molecular complexes which he studied, either were too stable, and consequently unable to fulfill the condition of being readily dissociated (such as the complex, benzaldehyde: trichloroacetic acid), or were too unstable (such as those whose existence is based uniquely upon the examination of solidification curves). In the latter case, the reactivity of the catalyst toward the auto-oxidizable substance is far too feeble to allow observation of its activity in the high dilutions which are used. In our opinion, the essential condition is the union of the catalyst with the active molecules, and not with the mass of molecules in the mean state. This latter condition would even be prejudicial, since the molecules held in a stable complex would be lost for catalytic activity. The ideal anti-oxygen would be one which was deprived of all reactivity toward average molecules of the auto-oxidizable substance, but would have a great power of attraction for active molecules. It would be exceedingly difficult to adduce experimental evidence of combinations of this type, and one must not be surprised that Taylor was able to discover only a rather vague relationship between the anti-oxygenic activity of a substance and its tendency to form molecular complexes with average molecules of benzaldehyde.

We would go one step further, in that we believe that no theory of anti-oxygenic catalysis can avoid the necessity of invoking a selective (if not exclusive) attraction of the anti-oxygen for at least one of the active members of the transformation (active molecules of oxygen, or of the auto-oxidizable substance, or of the first products of their union). One can readily see objections to any other explanation of the protective action of one molecule of hydroquinone upon forty thousand molecules of an auto-oxidizable substance. First, a protective action would obviously be impossible if the forty thousand molecules were able to react with oxygen simultaneously. Second, it is essential that as soon as one of the molecules enters into reaction, the anti-oxygen reach it surely and immediately, in order to act before an irreparable injury has resulted.

In bringing about an inactivation the anti-oxygen must not

hesitate between active molecules and the others, nor waste time in the formation of sterile combinations. It must act as a magnet to seek exclusively and rapidly, those molecules which are on the point of undergoing a definite transformation. *This result can only be obtained by a marked power of selective attraction, which we believe to be indispensable for anti-oxygenic activity.*

One can scarcely object to the concept that a reacting molecule may choose, among the mass of surrounding molecules, those which are in an activated state. In so doing, the reacting molecule would but imitate oxygen itself, which combines with active molecules to the exclusion of all others.

The need of another theory. Notwithstanding the interesting theories based upon a pure and simple inactivation of the auto-oxidizable substance, we do not believe that they can account for the mechanism of anti-oxygenic action except in certain special cases.

At the outset, they have against them the wide variety of anti-oxygens and auto-oxidizable substances. It would be necessary to suppose that such widely different substances as sodium iodide, sulfur, hydroquinone, thiohydracrylic acid, *et cetera*, act upon a single substance such as benzaldehyde, in modifying it in the same manner. It would be necessary to admit also, that the substances in such a varied collection as the following, benzaldehyde, acrolein, essence of turpentine, sodium sulfite and linseed oil, undergo the same transformations on contact with the same reagent, such as ethyl xanthogenamide. It is evident that one of these suppositions is as unlikely as the other.⁴²

An experimental proof that anti-oxygens do not act by the inactivation of auto-oxidizable substances would be highly desirable. One realizes the experimental difficulties which must be surmounted, when one considers that we are dealing with researches involving ephemeral molecules which are probably present in almost infinitesimal amounts (probably of the order of one per million, or even less).

⁴² In any case, the second objection would have less force against a theory based upon the inactivation of oxygen, since in all the infinite variety of auto-oxidative phenomena, this is the only thing which remains identical.

In one special case, through a combination of favorable conditions, we had the good fortune to be able to determine directly, whether or not an auto-oxidizable substance was inactivated by an anti-oxygen. The case was that of acrolein to which hydroquinone had been added.⁴³ We came to the definite conclusion that the inactivating effect of hydroquinone toward acrolein, if it existed was quite insufficient to explain its anti-oxygenic action. Although such a cause may intervene (which is possible, but would need to be demonstrated), there is certainly another which is much more important. Further, it would be extraordinary if we had just happened to fall upon an exception in the example just mentioned. In any event, since one cannot explain the action of hydroquinone upon acrolein as an inactivation of the latter, another theory is necessary, and it is this theory which we propose to discuss in the following paragraph.

Indirect Inactivation

A general theory of anti-oxygenic action: a process of indirect inactivation

From the debut of our researches we were occupied with the formulation of a mechanism to account for the peculiar action of anti-oxygens. Very early we conceived the following theory which has served as a valuable guide in the orientation of our investigations.

We suppose, with the majority of authors (Engler and Wild, Bach, and others), that auto-oxidation starts with the union of an oxygen molecule, O_2 , with a molecule of the auto-oxidizable substance A, giving rise to the peroxide $A[O_2]$, which we shall call the primary peroxide. Our notion of this combination differs considerably, however, from that of our predecessors. To us, this peroxide or first term of the successive transformations which an auto-oxidizable substance takes with oxygen, is formed with an absorption, and not a liberation, of energy. The peroxide results from the union of active molecules of A and O_2 , not of average molecules, and the union is effected with loss of very little energy.

From the standpoint of energetics the combination $A[O_2]$,

⁴³ Reference 25, III, and XII-XV.

formed by elevation of potential from the system $A + O_2$, must be placed toward the summit of the curve representing the phenomenon (see fig. 9). In the formula, oxygen is enclosed in brackets in order to indicate that it is present in an activated state. We shall follow the same designation for other oxides ($M[O_2]$ or $M[O]$) when we wish to indicate this particular state of oxygen.

On this basis we believed that anti-oxygens must act in catalysing the inverse reaction of the formation of the peroxide $A[O_2]$, that is, its destruction. In addition to the liberation of free oxygen from combined oxygen, it would be necessary that the reaction be very rapid and also complete (since one molecule of hydroquinone can protect forty thousand molecules of acrolein

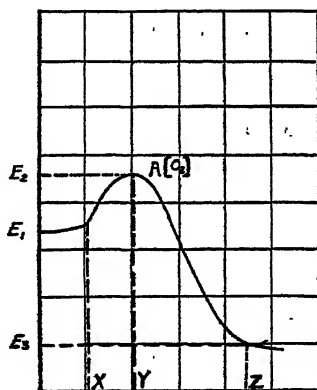


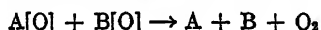
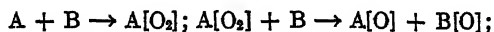
FIG. 9

from auto-oxidation), and we immediately thought of the mutual destruction of peroxides—well known reactions in which oxygen is almost totally and instantly liberated from such compounds.

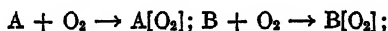
Thus we have come to the following explanation of the mechanism of the action of anti-oxygens:

We suppose that the peroxide $A[O_2]$ oxidizes the anti-oxygen B, with the formation of a peroxide $B[O]$, while it is itself transformed into another peroxide $A[O]$. The two peroxides $A[O]$ and $B[O]$ are antagonistic, and mutually destroy each other, (as has been observed for numerous antagonistic peroxides)

with regeneration of the three original molecules, $A + B + O_2$, in their original state. This notion of anti-oxygenic action is summarized in the three equations which follow:



We should not exclude the hypothesis of a direct auto-oxidation of the anti-oxygen; in this case, we would have the following cycle:



It will be noted that A and O_2 have been taken from the state of *activated molecules* at the moment of their combination, and returned to the mixture in an inactivated state. Our so-called negative catalysis is thus, in reality, a positive catalysis with its normal effect, since it favors a drop of potential. This action corresponds to the portion of the curve (fig. 9) projected in XY , where the potential energy falls from the value E_2 , to the value E_1 .

We have thus a rational solution of the paradoxical problem which was presented at the opening of this section; i.e., that of an impelling force corresponding to a positive catalysis, which has the effect of leaving the stone undisturbed on the side of a hill. It suffices to suppose that the resistance which opposes accelerated descent is none other than irregularities of contour. The stone must slide over each of these irregularities before being able to fall. Therefore, when the stone is at the summit of an irregularity, if there is a cause which facilitates its return to the original lower level, this corresponds to a true positive catalysis involving a loss of potential energy, and the stone will not proceed on its course downward. We have in this particular causation, the picture of a true positive catalysis of which the apparent effect on the general course of the reaction is negative.

This mechanism of indirect inactivation is essentially different than a separate inactivation of the auto-oxidizable substance or

oxygen, which was mentioned above. In these two inactivations, one considers that either the auto-oxidizable substance alone, or the oxygen alone, is inactivated by the activity of the anti-oxygen. In pure acrolein, for example, the concentration of active molecules would be diminished simply by contact with the anti-oxygen, and the same would be true for oxygen. According to our theory, on the contrary, acrolein could only be inactivated by anti-oxygens in the presence of oxygen, and oxygen could only be inactivated in the presence of an auto-oxidizable substance. In brief, by mutual interaction we simultaneously inactivate both oxygen and the auto-oxidizable substance.

This theory allows the prediction of numerous results, of which a certain number have already been found in good accord with experimental observations:

1. However strange it may seem, the power of preventing the action of free oxygen must belong to oxidizable substances. In fact, we have found this property among more than three hundred oxidizable substances (phenols, inorganic and organic compounds of iodine, sulfur, nitrogen, etc.). Let us recall in this connection, the remarkable example of phosphorus sesquisulfide, a highly oxidizable substance which acts as a powerful anti-oxygen toward benzaldehyde.

2. This catalytic property can be possessed exclusively by oxidizable substances. To our knowledge, there is not a single anti-oxygen which is not capable of oxidation.⁴⁴

3. The activity of an anti-oxygen is localized in the oxidizable portion of the molecule.

We have been able to demonstrate this in the case of sulfur.⁴⁵ For instance, dimethyl sulfide, $(\text{CH}_3)_2\text{S}$, hinders the oxidation of benzaldehyde, and this activity is due to the sulfur, which is the oxidizable portion of the molecule, and not to the methyl groups. This is shown by the fact that on oxidation of the sulfur in this molecule, by conversion to dimethyl sulfone $(\text{CH}_3)_2\text{SO}_2$, its oxi-

⁴⁴ Mittra and Dhar in 1922 made the independent observation that the auto-oxidation of sodium sulfite and of stannous salts was inhibited only by oxidizable substances.

⁴⁵ Moureu, Dufraisse and Lotte, reference 25, XVI.

dizability is suppressed, and we observed that its anti-oxygenic activity was destroyed at the same time, although the general structure of the molecule remained unchanged. Analogous results were observed with other substances containing sulfur and with oxidizable substances other than benzaldehyde.

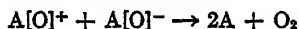
4. The catalytic activity of an anti-oxygen should increase with increase of oxidizability.

In accordance with this prediction we have observed, for example, that bromides are in general much less active than iodides, and that chlorides and fluorides are practically without action.⁴⁶

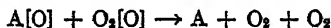
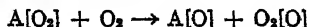
5. Every oxidizable substance should be able to act as an anti-oxygen under favorable conditions. This property would be a general one associated with all oxidizable substances; the favorable conditions would vary according to the nature of the substances selected.

In the case of any two auto-oxidizable substances that are placed in contact with each other, it should be possible to find conditions under which one may play the rôle of an anti-oxygen toward the other.

It is even possible to imagine a case in which a substance acts as an anti-oxygen toward itself.⁴⁷ One may suppose that the substance can give rise to two antagonistic peroxides, either directly or after a spontaneous transformation:



Since oxygen itself is capable of forming a peroxide (ozone, O_3 , is none other than a peroxide of oxygen, $O_2[O]$), it may act in certain cases as the anti-oxygen B:



⁴⁶ The oxidizability that we invoke here as an essential condition of catalytic activity, requires that an antioxygen have a certain reactivity toward oxygen. This is not entirely sufficient; other conditions dependent upon the nature of the auto-oxidizable substance and external conditions (temperature, etc.) must be fulfilled. We cannot yet state exactly what these conditions are, although we have many interesting observations on this subject.

⁴⁷ This prediction, and the one following, are illustrated by a subtle analysis, of the auto-oxidations of sulfur compounds and of phosphorus, made by Delepine. *Bull. soc. chim.* (4) 31, 782 (1922).

The auto-oxidation of phosphorus is an example of this kind. It is known that this auto-oxidation is prevented by an excessive concentration of oxygen. To us, oxygen plays the part of an anti-oxygen, according to the above scheme. Let us recall the well known fact that during the course of this auto-oxidation, formation of a peroxide of oxygen has been demonstrated by the formation of ozone.

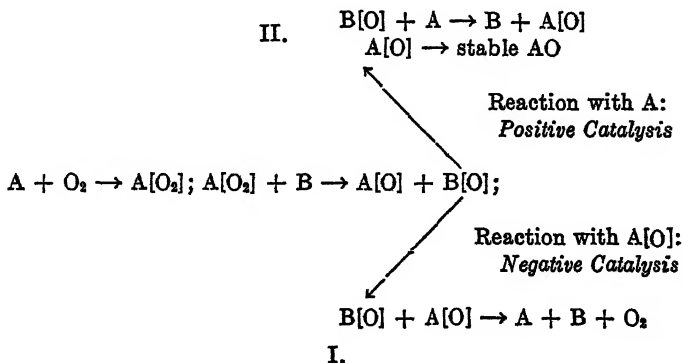
6. A logical development of this theory leads to the following conclusions: (i) the two inverse senses of catalysis, positive (pro-oxygenic action) and negative (anti-oxygenic action), are closely related to each other, and at the outset at least, they follow an analogous mechanism; (ii) the sense of catalysis is determined by experimental conditions, and a slight change of the latter may result in an inversion of the sense.

Let us reconsider the second phase in the scheme which we have presented for the mechanism of anti-oxygenic action. There are formed two transitory peroxides, $A[O]$ and $B[O]$, which are very reactive toward each other and are going to pass through a series of transformations. What are the possible transformations? We have described one according to which $A[O]$ and $B[O]$, being antagonistic, react immediately with each other, causing a mutual reduction with liberation of oxygen. This is anti-oxygenic action. But one must realize that other reactions may also occur with the same ease. In particular, let us remark that the peroxide $B[O]$ is surrounded by a mass of molecules of A , which are strong reducing agents. $B[O]$ may thus be strongly drawn toward the reaction $B[O] + A \rightarrow B + A[O] \rightarrow AO$ (stable).⁴⁸ B , which is liberated after this cycle of reactions, may then recommence in the same way and there results a positive catalysis of the oxidation of A under the influence of B . It is evident that B will act

⁴⁸ Among other reactions which may be imagined, we shall mention only the following, rather important one. The transitory peroxide $B(O)$, before acting upon $A(O)$ (negative catalysis) or upon A (positive catalysis), may tend to pass into a stable form (stable BO), in which case the molecule B which has undergone this transformation, is irreparably lost for catalysis. We have explained in this way certain characteristics of the phenomena of auto-oxidation; namely, coupled reactions (see reference 25 (XII) and exhaustion of the catalyst, which is a more or less well defined occurrence in all of our experiments.

as a positive or a negative catalyst, depending upon whether the peroxide B[O] prefers to act upon A[O] or upon A.

The following diagram indicates the two cycles:



The first two steps of the reactions are common to both modes of activity, since the sense of the catalysis is not determined until the third step is reached. The respective probabilities of the reactions I and II determine the sense of the catalytic effect; reaction I is favored by its velocity, reaction II by the concentrations.

With regard to reaction I, it may be said that reactions between antagonistic peroxides are rapid and complete, if we judge by such examples as the mutual decomposition of hydrogen peroxide and permanganates. In the latter we are dealing with stable peroxides that would be expected to be much less reactive than the transitory peroxides which we have formulated in the above diagram. This would lead us to expect greater velocities of reaction between the transitory peroxides A[O] and B[O], if such a thing is possible. Further, the probability of an increased velocity of reaction between the two antagonistic molecules A[O] and B[O], is increased by the fact that they are produced in the same reaction and in close proximity to one another, so that no time is lost in effecting contact of the two substances.

With regard to reaction II, it is clear that if the circumstances are such that B[O] separates from A[O] even for an instant before reaction, the chances for reaction between B[O] and A become

preponderant, since B[O] immediately finds itself in the zone of action of an enormous number of reducing molecules A, and this obviously gives to the reaction II the important advantage of concentration.

In principle, then, very slight changes may suffice to determine the sense of the catalysis. It is to be assumed that the two reactions can be effected simultaneously in the same medium, and the proportion of each will vary according to its inherent tendency to occur. The apparent sense of the catalysis (acceleration or retardation) will be the net effect, i.e., the algebraic sum of the effects of the two inverse catalyses.

What factors will determine the preponderance of one or the other of these two characteristic reactions, and consequently decide between an anti-oxygenic and a pro-oxygenic action? Manifestly, the experimental conditions play an important part. Let us attempt to analyze the situation. First, we must consider the nature of the substances present; namely, oxygen, common to all cases, an auto-oxidizable substance A, and a catalyst B, (either of the latter two extremely varied in nature). Other variables are temperature, light effects, concentration, and certain other characteristics of the media, of which certain ones are beyond our control, e.g., action of the walls of the vessels. It is quite certain that the individual nature of A and B is of great importance in the orientation of the phenomenon, but on account of the slight modification required to change this orientation, it is to be assumed that a knowledge of A and B will not suffice to determine the behavior of the system, and consequently other variable conditions may also play a decisive rôle.

A priori, it is impossible to assign definitely to any given catalyst B, the property of anti-oxygenic or pro-oxygenic activity. In practice, B will behave as a positive or a negative catalyst, according to the nature of the substance A with which it is in contact, and according to the conditions of observation of the action of oxygen upon the mixture of A and B.

Another source of complication is the fact that a catalyst may become altered during the course of the experiments, in which case one must expect that the observed facts may be even differ-

ent, since B will have been converted to a new substance B' which will react in place of B. In this case, if one does not have experimental indications of such an alteration, one will attribute to B the effects which are actually due to B'. In our opinion this is the explanation of certain modifications of the nature of catalysis that are produced in the course of experiments in which no apparent change has been made in any of the conditions; e.g., the same auto-oxidizable substance A, the same catalyst B, the same external conditions, the same reaction vessel, and the duration of contact as the only variable. Instead of a regular procedure, anomalies are observed in the oxidation curves, which may even go so far as an inversion of the sense of catalysis. Certain characteristic examples have already been mentioned in the first chapter. We have experimental evidence that this inversion of catalysis in the course of a single experiment is due to an alteration of the catalyst, in observing the behavior of iodine and a number of iodine derivatives toward acrolein. These catalysts produced first an anti-oxygenic action, which was followed rather suddenly by a more or less intense pro-oxygenic action, after an interval of time varying with the nature of the iodine compound used, and its concentration. In fact, with iodine compounds there was always observed at first a reddish yellow coloration (similar to that given by free iodine), and the pro-oxygenic phase did not occur until after complete decolorization of the liquid.

Let us add also, that the course of a catalysis may be modified by the alterations previously cited in connection with the exhaustion of a catalyst through oxidation into products which may themselves possess specific catalytic properties, or be without any activity. We have called attention to these facts apropos the passage of the thiol group, $-S-H$, into the disulfide, $-S-S-$, and vice-versa, in the case of certain sulfur-containing catalysts.^{25,39}

The close relationship of the two inverse catalyses, and an easy inversion of the sense of the catalysis, are two unexpected but ineluctable consequences of our theory.

No one would challenge the theoretical and practical impor-

tance of these deductions, and the experimental demonstration of these points was of fundamental importance. An abundance of facts reported in the first chapter serves to establish these concepts with certainty.

One realizes from the foregoing that it would be exceedingly difficult in practice, to foresee the sense of the effect produced by this veritable "oxidizable impurity" which acts as the catalyst B; either an acceleration or a retardation of auto-oxidation may result from its presence. We have not so far succeeded in developing a rule which is sufficiently general to permit predictions of the sense of the catalysis. It has even happened, as one might theoretically foresee, that the same catalyst may behave toward the same oxidizable substance, either as an anti-oxygen or a pro-oxygen, depending upon the experimental conditions. We may remark that the useful acquisition, both from a theoretical and a practical standpoint, is the simple fact that we are warned of the possibility of such an inversion.

We are pursuing our experiments in a systematic manner, and perhaps they will lead to the formulation of rules that may be applied to new cases for the prediction of their behavior.

In résumé, one can see that our general conception of the mechanism of anti-oxygenic action is in satisfactory accord with the experimental observations. Our theory has not only enabled us to account for all the facts already known, but also to foresee a multitude of others, which but for it, would have been entirely unexpected.⁴⁹ We may be permitted to emphasize the fact that this theory has allowed a passage without transition, from the phenols to other categories of anti-oxygenic substances of an entirely different nature, such as inorganic and organic compounds of iodine, sulfur, nitrogen, et cetera.

We believe also, that our conception will be easily able to account for numerous facts which are still regarded as mysterious, and we have already been able, in diverse cases, to propose a rational interpretation of existing facts.

⁴⁹ It may be pointed out that certain other scientists (A. Gillet and others) have already successfully applied our conceptions to problems in which they were interested.

CONCLUSION

To characterize this long study in a few words, perhaps one would say that it abounds in surprises and improbabilities. To this we reply, that in science even more than in the theatre, according to the celebrated quotation of Boileau: "*le vrai peut quelque fois n'être pas vraisemblable*" (the truth may sometimes not seem probable).

Is there a man of thought and reflection, to whom Nature does not appear vastly more marvelous, and stranger than all that which he may draw from the most profound depths of his own knowledge?

Let us recall our beginnings—by what tortuous ways we were led over a narrow and obscure path to the negative catalysis of auto-oxidation—how, in the search for a directing light, we built up a theory which made possible not only a facile interpretation of facts already known, but also the prediction of a number of others, which without it, would have been entirely unexpected. We have pointed out what significance this work has in the diverse fields of speculative science, industry, and biology.

It is not surprising to see our horizon thus continually enlarging. We live in oxygen, the essential agent of respiration; oxygen bathes all the bodies and objects which surround us, and its great reactivity is well known. If then one were to effect by any means, a moderation or an excitation of the universal avidity of such an element, would there not result new perspectives in the chemistry of Nature, living and dead?

The general phenomenon of auto-oxidation appears to be favored by certain agencies and deterred by others. A continual struggle goes on between the positive and negative catalysts of this reaction, and anti-oxygenic activity appears along with the action of chlorophyll, the regenerator of oxygen, as an equilibrating factor in the movements of oxygen on the surface of the earth.

We may remark further, concerning our theory, that it constitutes a direct application by way of experimental research, of the idea of active molecules. It appears difficult to arrive at a conception of anti-oxygenic action without a belief in the

existence of active molecules, just as it was difficult before the introduction of that concept, to have a correct idea of the slowness of reactions. In this way, the experimental facts upon which our theory rests may be regarded as experimental evidence of an entirely different order, for the reality of chemical activation of molecules.

Along another line of thought, it seems reasonable to suppose that in addition to anti- and pro-oxygenic activity, one may encounter corresponding effects with other elements, or even with certain compounds; for example, anti-chloric, anti-sulfic, anti-plumbic activities. Certain phenomena are already known that could be placed under the above terms. Is it not to an anti-chloric or an anti-bromic activity of oxygen that the retarding action observed in certain reactions may be referred? Without doubt these observations may be profitably used in diverse fields of pure and applied chemistry.

The domain is enormous. The more one reflects, the more one is persuaded that the discovery of traces of impurities and the study of their influences will give us a true understanding of a multitude of chemical phenomena, if not to chemical reaction itself, envisaged in its most general aspect. No doubt in work of this kind, the most fruitful light will come from precise data concerning the physico-chemical structure of molecules, which will come to us chiefly through studies of energetics, utilizing the methods of photochemistry and thermochemistry.

What great questions to resolve! What vast fields to cultivate! The imagination takes flight. . . . Who knows? After all its magnificent conquests, may not the science of chemistry, due to the study of catalysis, inaugurated by Davy and Berzelius, and reanimated after half a century of quiescence by Sabatier and Senderens—may it not again be but as at the dawn of a new era? But let us pause. For on such a delicate subject, as Dumas remarked, one always risks saying too much, however little one says. This much is certain: that the domain of the unknown is without limit in extent or depth, and that our knowledge will never equal our desire to know nor our joy to discover.

THE PROPERTIES OF SURFACE FILMS ON LIQUIDS

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EARLY OBSERVATIONS

It was not until about 1890, that systematic attempts were made to distinguish clearly between the properties of water surfaces when clean, and when covered by a film of contamination. It had been known that oil films on water diminish the surface tension, and also tend to calm wave motion; but there was not enough knowledge of the properties of surface for it to be easily ascertained whether a given surface of water was clean or not. In 1890 Rayleigh (1) measured the amount of olive oil necessary to check the motions of camphor, which take place on a clean surface, though not on a contaminated one; and he also measured the amount of the depression of the surface tension of water caused by the minimum quantity of oil required to stop the camphor movements (3,5). The diminution of surface tension at this point is about 16 dynes per centimeter; calculating from Rayleigh's determination, the area per molecule of the oil, at this point, was 100 sq. Å.U.,ⁱ assuming as is not far from the truth, that the oil consists wholly of triolein. This is in excellent agreement with the most recent measurements, which give 97 sq. Å.U. at this depression of surface tension, for triolein.

In the same year Rayleigh (2) found that Plateau's observation (4), that the surface viscosity of water is greater than the internal viscosity, is not true of a clean water surface, but that any excess viscosity is due to the presence of a surface film which can be cleaned away. In 1892 (6) he found that certain anomalies in the reflection of light from water surfaces were also due to surface films of contamination.

ⁱ 10^{-18} sq. cm.

Miss Pockels, in 1891 (7), showed that a film on a water surface may be mechanically removed, or rather concentrated in any desired part of the surface, leaving the remainder clean, in just the same manner that fallen leaves on a swimming bath may be swept up by a floating plank pulled from end to end. By the use of barriers working on the surface of water contained in a trough filled to the brim she was able to vary the surface available for a given amount of oil present as a film. She found that when the area available exceeded a certain amount, there was no perceptible diminution of surface tension, but as soon as the area was diminished below this, a rapid fall of surface tension began. The damping of wave-motion was closely connected with the area of the surface film; and the so-called solution currents from a solid body dipped into the liquid, the streams of matter leaving the solid and spreading out along the surface, made visible by dusting the surface with a light powder, might be stopped by reducing the area of the surface film.

THE CONCEPTION OF SURFACE PRESSURE, AND OF THE FILMS
AS ONE MOLECULE THICK

In 1899, Rayleigh (5) confirmed Pockels' observation on the manner of variation of the surface tension with the area of the film, and suggested that at the point of the first fall in surface tension, the molecules of the film were just crowded together into contact, to form a layer one molecule thick. He wrote:

. . . . at what point will an opposition to contraction arise? The answer must depend upon the forces supposed to be operative between the molecules of oil. If they behave like the smooth rigid spheres of gaseous theory, no forces will be called into play until they are closely packed. . . . If we accept this view, we conclude that the first drop in surface tension corresponds to a complete layer one molecule thick, and that the diameter of a molecule of oil is about 1.0μ An essentially different result would seem to require a repulsive force between the molecules, resisting concentration long before the first layer is complete.

This suggestion involves two points of great importance; that the molecules of oil can be likened to small hard floating

bodies, and that the diminution of surface tension is nothing more than the force required to compress these floating molecules close together against the repulsive forces between them.

Rayleigh did not elaborate these points, but they have proved fundamental, and indispensable, to the further development of the theory. That the molecules of oil may be legitimately considered as floating objects follows from their insolubility in water. The surface pressure, or outward force exerted by a surface film upon a barrier bounding it, is of the same nature as the outward pressure which would be exerted by a "film" composed of visible floating objects. There must obviously be some such pressure caused by any floating objects which possess size and shape, on a barrier which allows the water molecules to pass, but not those of the film; and any float which blocks the whole surface must act as such a "two-dimensional semipermeable membrane." So far, this point of view seems obvious and legitimate. But some difficulty has been caused in its acceptance, by the very strong tendency which existed during last century, and still persists to some extent, to regard the resistance which the surface of a liquid offers to any increase of area, as due to a structure present in the surface, which has some of the properties of an elastic skin enclosing the liquid. The contractile force of such a skin is equal to the surface tension of the liquid, according to those who regard it as a reality; and since the films diminish the surface tension of the water, the molecules of oil, on that theory, must operate in some manner to impair the strength of the surface skin.

Are we to regard the force on a float bounding an area of the surface covered by a film, as due to the outward pressure of the film molecules, or to the difference between the outward tension of the clean water surface on one side, and the smaller inward tension of the dirty surface on the other? Apart from the pre-disposition to regard the surface of a liquid as in a "state of tension," which is a natural, but not a logical, result of the general use of the word "surface tension" to express the work which must be done per unit area of fresh surface formed, the idea of surface pressure has so much in common with other branches of

Physics that it appears unquestionably correct. It might be asked, how much progress should we have made, by regarding the force on the walls of a vessel containing gas, as the difference between the tension of the vacuum outside the vessel, and the diminished tension of the "contaminated vacuum" inside? Attempts to regard the force on the boundary of a film as due to the difference between two tensions, have not led to any tangible theory of the molecular structure of the films, whereas abandoning the idea of a contractile skin, and using the idea of an outward pressure of the film molecules—a two-dimensional osmotic pressure on the perfect semipermeable membrane which the float forms for the insoluble films—very complete analogies have been discovered between the arrangements of the molecules in the two dimensions of the films, and the ordinary forms of matter in bulk. The ideas of surface pressure, and of a contractile skin, are mutually exclusive; and if the contractile skin theory is wrong in the application to properties of surface films, it must also be wrong in its application to all other phenomena of Capillarity. Many writers have warned against the contractile skin theory; in a recent note (8) the author has attempted to show that the so-called proofs of the existence of such a skin are invalid, and that satisfactory explanations of the phenomena of Capillarity can always be obtained without its use; and also that the various forms assumed by the contractile skin theory in the literature are generally contradictory when applied to other phenomena than the single one for which they were invented. Much remains to be done, in developing the theory of Capillarity on the usual lines of molecular Physics; and the author is convinced that all remnants of the idea of a contractile skin in the surface of liquids must be cleared away before progress can be made in this direction.

The surface pressure is quantitatively equal to the diminution in the surface tension of the surface by the film, for if the barrier bounding a film which exerts a pressure F is moved inwards so as to diminish the area by 1 sq. cm., work F ergs must be done; but if the surface tension of the clean surface is γ and that of the film covered surface is γ' , the mechanical work is $\gamma - \gamma'$, since

the surface tension is only the work necessary to increase the area of a surface by 1 sq. cm. It is frequently necessary to determine the surface pressure by taking the difference between the surface tension of the film covered, and of the clean surfaces.

DEVELOPMENTS OF THE MONOMOLECULAR THEORY

The rise of surface pressure after the molecules first come into contact was soon found not to be always quite abrupt, and this caused some doubt as to the films being always 1 molecule thick. Marcelin (9) for a time considered that the area to which it is necessary to compress the films of several oils, to bring them into equilibrium with drops in bulk on the surface, is so nearly half the area of maximum extension of the film, at which the first surface pressure is developed, that the point of equilibrium with the drops is that at which the film is 2 molecules thick. Devaux (10) considered it possible that the films were more than 1 molecule thick, when compressed. The ratio of the areas at the first appearance of surface pressure, and at equilibrium with the drops, is not usually however two to one; figure 2 shows that for the saturated fatty acids it is not far from 1.01 to 1, and it would not be difficult to find substances in which it would be five to one or even more, according to the sensitivity of the means used to determine the first surface pressure.

In about five thousand experiments on films of long chain aliphatic substances, the author has never found a case of a film more than one molecule in thickness. When the molecules leave the surface, to mount on one another, as they must do when the area is sufficiently reduced, they pile up into aggregates many thousands of molecules thick, which are usually visible to the eye. That this is likely to be the case is shown, when the forces on individual molecules are considered. They are anchored to the water by an attraction perpendicular to the surface, and the film, in the closer stages of packing, is further stabilized by the lateral attractions between the long chains, which are often packed closely side by side. For a single molecule to leave the film, and lie on the top, would require the overcoming

of both the perpendicular and side by side attractions; but if a number are expelled together, the side by side cohesions are not disturbed, and even the work of overcoming the perpendicular attractions is diminished by the attractions which the molecules exert on one another as they come together outside the film.

LANGMUIR'S WORK

The next considerable advance in our knowledge of the structure of surface films was made by Langmuir in 1917 (11). He accepted the monomolecular idea completely; making numerous experiments, measuring surface pressure directly by observing the outward pressure on a float in the surface, and also the area per molecule by measuring the area of the films and the quantity of substance put on, concluded that the films of insoluble fatty substances with long chains are fairly closely packed on the surface, in some cases so closely packed as to have the properties of a solid substance on the surface. With the closest packings, the area of the film was independent of the length of the molecule in any one homologous series of compounds. The area per molecule in this closest packing was about 21 sq. Å.u., a value fairly close to that obtained by assuming the film to be of the same density as the material in bulk, the long chains oriented vertically and the distance of the carbon atoms above each other 1.5 Å.u., the distance apart of the carbon atoms in the diamond. The independence of area and length of the chain alone points very strongly to a vertical orientation of the chains. There were some complications, the area of some of the films being roughly double that of the closest packing; Langmuir did not elucidate these, but later work has shown that they can be explained without conflicting with the essentials of his view.

Langmuir also dealt with the structure of the films adsorbed at the water-air surfaces of slightly soluble fatty acids, and many other substances. Direct measurement of the outward pressure of the film molecules cannot be made by a float in the surface in this case, as the film molecules can pass under the float by dissolving in the water. But the surface pressure is the dif-

ference between the surface tension of the solution with the adsorbed layer formed, and that of the pure solvent, or more strictly that of the solution if it were possible to measure surface tension so rapidly that the adsorbed layer had not been formed. Measurements of the surface tension of the solution therefore provide a measure of the surface pressure of the adsorbed film.

The area per molecule may be found from Gibbs' adsorption equation, when the surface tensions are known over a sufficient range of concentration of solution. The equation may be exactly written in any of the following forms (Γ is the number of molecules adsorbed per sq. Å.u., A the area per molecule, γ the surface tension, F the surface pressure, μ the potential of the solute, a the activity, c the mol fraction or concentration, R , is 1.372):

$$\frac{1}{A} = \Gamma = - \frac{d\gamma}{d\mu} = \frac{dF}{RT d \ln a} \quad (1)$$

and approximately in the form

$$\frac{1}{A} = \frac{dF}{RT d \ln c} \quad (2)$$

The variation of $\log c$ or $\log a$ with F is determined experimentally by measuring the surface tensions of the solutions at various concentrations, and the area per molecule found from the slope of the curve relating these quantities.

Langmuir reviewed a large amount of data in the literature on the surface tensions of aqueous solutions, using the approximate form of Gibbs' equation. When the solutions were sufficiently dilute, the relation $FA = RT$ holds. This is precisely analogous to the relation between pressure and volume in an ideal gas or ideal solution. Indeed this is to be expected, since the surface is a solution in two dimensions, and any float which could prevent the surface film molecules passing, allowing the water molecules to pass freely, would be an ideal two-dimensional semipermeable membrane. We have seen that such a float, or perfect barrier to film molecules, is realisable experimentally in the case of the insoluble and non-volatile films, so that the surface pressure F is clearly a surface osmotic pressure.

When the solutions become more concentrated, the relation $FA = RT$ does not hold, but the adsorption increases until with large concentrations it is nearly constant, at 31 sq. Å.u. per molecule.

Langmuir considered that the insoluble films which he investigated by the float method were analogous to two-dimensional solids and liquids, and the adsorbed films in the dilute state to two dimensional gases. He predicted a series of phenomena completely analogous to evaporation, as a connecting link between these two states. This prediction has been fully verified, quite recently. Langmuir also applied the theorem of kinetic theory, which gives the relation between the concentrations of a gas in two regions differing in potential energy, to the equilibrium between the surface and interior of the solutions, and obtained information as to the orientation of the molecules in the gaseous condition of the films. This will be explained later.

THE PRESENT POSITION. GASEOUS FILMS

Owing to the small size of the molecules, the normal state of the films is one in which the thermal agitation plays a predominant part. The pressure on a barrier, when the floating objects are small enough, will be a series of impacts from the moving objects, not a steady pressure. With the longer chain compounds studied by Rayleigh and Langmuir, there was much cohesion in the films, so that sometimes the effect of the thermal motions could be neglected, and the size and shape of the molecules were the predominant factors in determining the properties of the films.

When there is a negligible cohesion between the film molecules, and the molecules are small in comparison with the area available in the surface, the average kinetic energy in each degree of freedom of the surface, $\frac{1}{2} RT$ for each molecule, operates wholly to produce pressure on the barriers, that is, the surface pressure. Then it may be shown by the methods of kinetic theory that $FA = RT$. The same equation may be deduced thermodynamically by a combination of Gibbs' adsorption equation, with Langmuir's kinetic theory of adsorption, or some equivalent theory

which will account for the fact first observed by Traube (12), that in dilute solutions the surface pressure is proportional to the concentration in the bulk of the solution.²

Films in which the relation $FA = RT$ is approximately obeyed are called "gaseous" films. Figure 1, which is compiled from direct measurements by Jessop and the author (19), in the case of the acids from C_{12} upwards, and from Schofield and Rideal's calculations, using Gibbs' equation in its exact form and v. Szyszkowski's (24) and Frumkin's (25) measurements of the surface tension of the aqueous solutions of the normal acids from C_4 to C_{12} , shows the variation of the product FA with F . As with gases and vapors; this method of plotting emphasizes the departure from the perfect gaseous state. The equation $FA = RT$ is represented on the diagram with sufficient accuracy by the horizontal line $FA = 400$, since all the measurements are within 10° of 18° . All the acids approach the perfect gaseous condition at very low pressures; but in the case of the acids above C_8 , they do not approach closely to this condition above pressures of one dyne.³ The curves are dotted where the absence of experimental data at the present time makes their actual course a matter of extrapolation. The curves resemble so closely the PV - P isothermals of a gas-liquid system at different temperatures, that it is reasonable to continue the extrapolation down to zero pressure along the line $FA = 400$, when this line has already been reached at higher pressures. At present the direct method of measuring surface pressures, by recording the actual force on a float, is much more accurate than the deduction of surface pressures by subtracting the surface tension of the film-covered surface from that of the clean surface; the direct and the indirect methods have both been applied to the C_{12} acid, almost identical results being obtained on the rising portion of the curve.⁴

² For an elaboration of this theory, the reader should consult (19, p. 424, footnote) and Schofield and Rideal (22).

³ Pressures will be recorded as so many "dynes," meaning dynes per centimeter.

⁴ The points determined from surface tension measurements and Gibbs' equation are marked Δ , and those by measuring the force on a float X . With the C_{12} acid, both methods are working under unfavorable conditions, owing to the slight solubility of the acid. The agreement is better than could be expected. Probably

The good agreement between the two methods is an excellent verification of Gibbs' equation, and the fact that the change in the curves, as the hydrocarbon chains are lengthened, is gradual and without any unusual feature where the direct method of determining surface pressure and area is substituted for that based on Gibbs' equation, is striking evidence of the equivalence

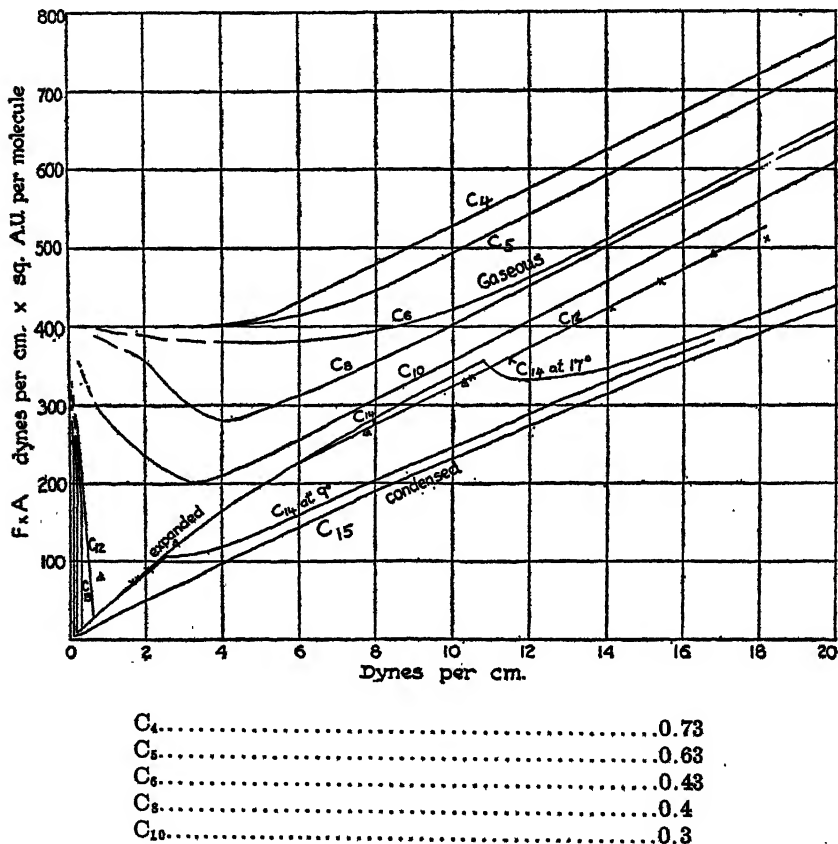


FIG. 1. GASEOUS FILMS

the curve for the C₁₄ acid is more accurate than that for the C₁₂, and it was to be expected that the C₁₂ curve would lie, if anything, slightly above that for the C₁₄ acid.

The experimental methods are described at the end of this paper.

of the two methods; it also proves that the adsorbed films, like the insoluble films, are 1 molecule thick.

The films of all the acids shorter than C_{12} are quite definitely gaseous in character; that is, their F - A isothermals correspond to the P - V isothermals of actual gases. For the acids shorter than C_6 , above 10 dynes per centimeter, the principal deviation from the perfect gas laws is due to the size of the molecules; an equation of the same form as Amagat's equation of state for gases under high pressures holding within the accuracy of experiment.

$$FA = FB + RTx \quad (3)$$

B is a constant, equal to 25 sq. Å.u. practically the same for all the acids C_4 to C_{10} ; it is the limiting area to which the films can be compressed. x has the following values; it decreases with increasing cohesion between the molecules.

At lower pressures, and especially with the longer chains, phenomena due to strong cohesion between the molecules appear. The curves of C_{10} , C_{12} , and C_{14} follow very closely the isothermals for carbon dioxide at various temperatures on either side of the critical point. The C_{12} curve is just above the critical point; this region is better seen in the curves of (19), figure 1. C_{18} is the first acid which shows a definite vapor pressure, and all the acids above this also show a vapor pressure, which becomes very small above C_{15} . The vapor pressures of the fatty acids, on dilute HCl (which is used as more reproducible results are obtained, and less trouble experienced from solubility, than on distilled water) are, C_{18} , 0.30; C_{14} , 0.18; C_{15} , 0.10; C_{16} , 0.04 dynes.

Similar evaporation phenomena have also been studied in the series of ethyl esters, primary alcohols, and nitriles, the main features being the same in all cases. The "critical pressures" are never greater than a few tenths of a dyne, and there are some differences between the films on the liquid side of the vapor pressure region, which will be discussed later.

WORK OF ADSORPTION. ORIENTATION OF MOLECULES IN GASEOUS FILMS

It is possible, as Langmuir showed, to calculate the work done in bringing a molecule from the adsorbed film into the interior of the solution against the surface forces, when the solution and the adsorbed film are both dilute. Considering the adsorbed layer, and the interior of the solution, as two separate solutions of different concentration, then Boltzmann's theorem of the relation between the concentration of the molecules in two different regions, where their potential energies differ by λ , states that

$$\frac{k\Gamma}{c} = e^{\frac{\lambda}{RT}} \quad (4)^*$$

$k\Gamma$ is the concentration in the adsorbed layer, and c that in the bulk of the solution. This equation is accurate only in the limiting case of infinite dilution, when the volume filled by the molecules is negligible in comparison with that of the region under consideration.

By Gibbs' equation

$$\frac{\Gamma}{c} = \frac{1}{RT} \frac{dF}{dc} \quad (5)$$

hence

$$\lambda = RT \ln \frac{k}{RT} \left(\frac{dF}{dc} \right)_0 \quad (6)$$

$\left(\frac{dF}{dc} \right)_0$ being the limiting slope of the surface tension—concentration curves at extreme dilution. We have seen already that the ratio $\frac{F}{c}$ becomes constant or nearly so at attainable dilutions.

* This equation may be more familiar under the form

$$\lambda = RT \ln \frac{k\Gamma}{c}$$

λ is the maximum work obtainable in taking a mol of the substance from the solution to the surface layer.

If an assumption is made as to the thickness of the surface layer, the concentration of the solute in the adsorbed film may be calculated and the constant k found. This is a rather arbitrary proceeding in our present state of knowledge; but in the case of any one homologous series we may assume as a first approximation that the thickness of this layer is the same, and therefore k the same, for each member of the series. It then becomes possible to calculate the increase in the work of adsorption as one CH_2 group is added to the chain of the molecule. By (4), in this case, when λ_n and λ_{n-1} are the energies of adsorption of successive members of the series, with n and $n-1$ carbon atoms,

$$\lambda_n - \lambda_{n-1} = RT \ln \frac{\left(\frac{\Gamma}{c}\right)_n}{\left(\frac{\Gamma}{c}\right)_{n-1}} \quad (7)$$

Szyszkowski (24) found that the surface pressure of solutions of the fatty acids from C_3 to C_6 could be approximately represented by the equation.

$$F = B\gamma_0 \log_{10} \left(1 + \frac{c}{\alpha}\right)$$

B being a constant having the value 0.411 for all the acids, and α a constant for each acid, for C_3 , 0.165; C_4 , 0.051; C_5 , 0.015; C_6 , 0.0043. The ratio of the values of α for successive members of the series is very close to 3.4 in each case. γ_0 is the surface tension of water, 72.8 dynes at 20° .

Differentiating,

$$dF = 0.434 B\gamma_0 \frac{dc}{c + \alpha}$$

and combining with Gibbs' equation in its approximate form (2),

$$\Gamma = \frac{0.434 B\gamma_0}{RT} \frac{c}{c + \alpha} = \frac{13.0}{RT} \frac{c}{c + \alpha} \quad (8)$$

For very dilute solutions, when c is small compared with α , this becomes

$$\frac{\Gamma}{c} = \frac{13.0}{RT \alpha} \quad (9)$$

Hence for the acids C_3 to C_6 , the ratio $(\Gamma/c)_n \div (\Gamma/c)_{n-1}$ is 3.4, and from (7),

$$\lambda_n - \lambda_{n-1} = 2.303 RT \log_{10} 3.4 = 4.91 \times 10^{-14} \text{ ergs per molecule or 710 calories per gram molecule}$$

The significance of the experimental fact that the ratio of the α 's in Szyszkowski's equation for successive members of the series is always 3.4, is that the energy of adsorption of each successive CH_2 in the chain of the molecule is constant and equal to 710 calories per mol. This constancy can only mean that each CH_2 group occupies a similar position in relation to the surface as all the others, and this can only be the case if the chains are lying flat on the surface.⁶

The proof that the chains are lying flat in the gaseous films only exists for the C_3 to C_6 acids, but the curves of figure 1 form such a continuous series that probably *in all gaseous films, the chains lie flat on the surface.*

By assuming the thickness of the surface adsorbed layer to be 6 Å.u., Langmuir calculated the work of adsorption for a large number of compounds. This could be expressed by the formula

$$\lambda = \lambda_0 + 625n \quad (\text{calories per mol})$$

λ_0 being constant for each homologous series, depending on the

⁶ The assumption that the thickness of the adsorbed layer is the same for successive members of the homologous series may of course be incorrect, but any possible error does not seriously impair the above argument that the chains are lying flat. Suppose that the layers are proportional in thickness to the length of the molecule; then the k 's in equation (4) will be inversely proportional to the lengths of the molecules, and the difference between the energies of adsorption will be less than those calculated from equation (7) by $RT \ln \frac{n}{n-1}$. The difference between $RT \ln \frac{n}{2}$ and $RT \ln \frac{n}{3}$ is negligible in comparison with the difference in the work of adsorption, $RT \ln 3.4$.

nature of the end group, and n the number of carbon atoms in the molecule. The values of λ_0 were

	END GROUP	λ_0
Monobasic acid.....	-COOH	437
Primary alcohol.....	-CH ₂ OH	575
Primary amine.....	-CH ₂ NH ₂	600
Ester.....	-COOR	470
Amide.....	-CONH ₂	-510
Ketone.....	RCOR'	295
Dibasic acid.....		-700

A double bond in the molecule decreases λ_0 by about 400 and every additional hydroxyl group in an acid decreases it by about 800. The value 625 for the coefficient of n is fairly near that of 710 previously obtained for the work of adsorption of a CH₂ group in the fatty acids, and indicates that the CH₂ groups in all cases lie in the surface, long chains lying flat; of course all these films are of the "gaseous" type.

Much further information might be gained by an accurate study of the influence of chemical constitution on the value of λ . The chief desiderata are a more accurate means of measuring small depressions of surface tension, and more data as to the assumptions made in assigning values to the thickness of the surface layer; the latter may be difficult to obtain, λ being the decrease in potential energy when a mol passes from the interior to the surface of the solution, a small value indicates high polarity, or tendency to dissolve in water. Langmuir pointed out that although hydroxyl and carboxyl groups increase the polarity, the polar character does not appear strictly additive, but two adjacent active groups enhance each other's polarity.

CONDENSED FILMS

When the cohesion between the molecules is great enough, and the temperature low enough, the molecules pack very closely, into "condensed" films. In these, the thermal agitation of the molecules is insufficient to permit them to have independent translatory motion along the surface, and the film

behaves as a coherent whole. This does not mean that experimentally it is possible to apply a tension to such a film along the surface; at least no attempt to do so has yet resulted in evidence that the film could support a tension. But it frequently happens that the film can resist a shearing stress in the plane of the surface, thus possessing surface rigidity. Condensed films, even those of the closest packing, may be either solid or liquid, or highly viscous but still liquid. Quantitative work on the surface rigidity of films is at present lacking. From qualitative observations, there is no doubt that there is sometimes a genuine rigidity; the author has sometimes had a film strong enough to resist a pressure of 2 or 3 dynes per centimeter, uniformly applied at one end, when there was no support at the other, the film being merely supported by the friction against the sides of the trough, 14 cm. wide. It is rare to find a definite "melting point" in the films, when the solid film suddenly loses rigidity and becomes liquid. One such case is known, with the acetamides (18). As a rule it appears as if there is considerable resistance to complete solidification, and possibly considerable "supercooling" even when parts of the film are solid. The molecules can only move to their equilibrium positions in the two dimensions of the surface.

The characteristics of condensed films are close packing, small compressibility, and negligible variation of area with temperature. When one chain is present in the molecule, the area is almost always between 20 and 30 sq. Å.u. per molecule. There are however wide variations among condensed films in area and compressibility, as the F - A curves of figure 2 show. These curves are of the following substances; the lower portions below about 2 dynes are dotted, as most of the data were obtained with the apparatus in which air jets disturb the equilibrium of the balance slightly (see the final section of this paper), and the lowest portions so determined were not reliable.

- I. Acids on distilled water (final curve); amides; di- and triglycerides (areas per chain, not per molecule); ureas above the transition temperature; acetamides below transition temperature.

- II. Ethyl, methyl, and allyl esters of saturated acids.
- III. Alcohols.
- IV. Acids on dilute HCl or H_2SO_4 .
- V. Iso-oleic acid on dilute HCl.
- VI. Ethyl iso-oleate.
- VII. Hexadecyl phenol and allied substances.
- VIII. Ureas below the transition temperature.
- IX. Nitriles.
- X. Final curves of the longer chain α bromo-acids, on dilute HCl.
- XI. Bromo-palmitic and bromo-margaric acids on HCl.
- XII. Acetamides above the transition temperature.

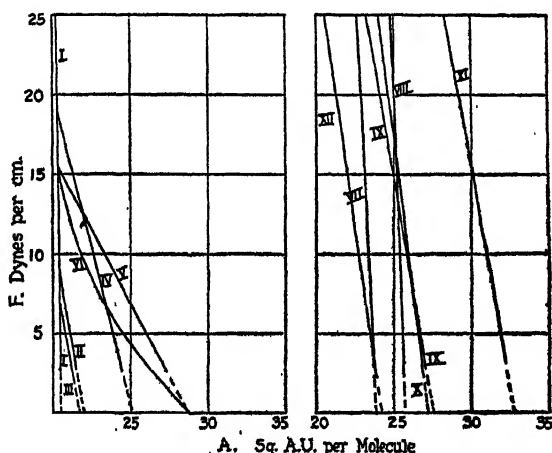


FIG. 2. CONDENSED FILMS

The data for these curves and some additional information is in (13-18).

There is practically no effect in most of the films, due to the length of the hydrocarbon chain, provided it is long enough to provide sufficient cohesion to keep the molecules in the condensed film. In the case of the bromo-acids there is however a definite difference in area between the 16 and 17 carbon acids, and the 18 and 21 carbon acids. The pair with the longer chains pack more closely than the pair with the shorter. In the aldoximes (17) and possibly also in the alcohols, there is a slight al-

teration in the curves, with length of chain. But these are minor differences, of which the different packings possible through the lower ends of the molecules being larger in cross section than the upper, may be the cause. The broad fact remains that the area is independent of the length of the molecules; in the case of the acids and alcohols, this has been verified for lengths from 14 to over 30 carbon atoms. The molecules are therefore oriented perpendicular to the surface.

The evidence that the polar group at the end of the molecule is the one directed downwards is as follows. It is only those long chain compounds with a water soluble group, that is a group which with a very short chain confers solubility on the molecule, which form stable films. The hydrocarbons, alkyl bromides and iodides, and the methyl ethers, do not form films at all, but the molecules remain in aggregates on the surface. Also if a large group is attached to the polar group, the adhesion of the film to the surface is destroyed; e.g., benzyl palmitate, palmitic anilide, cetyl palmitate, do not form films, simply because the polar group is prevented from approaching the water closely enough (16).

The curve of figure 1 is found with a large number of different types of compounds which have nothing in common except the long hydrocarbon chains. Some of these compounds only reach this curve at fairly high compressions, showing a definite lower portion, which is in all cases except that of ethyl iso-oleate a straight line. Areas smaller than those of curve I have never been found. It is obvious that the long chain compounds cannot pack together side by side to a smaller area than that of the long chains packed closely, therefore the curve I indicates that the long chains are touching each other in the closest packing. Where, as with curves II to VI, there is a definite lower portion of the curve, which cuts the abscissa at a greater area than curve I, the area at the foot of this lower portion is the cross section of the molecule at the widest point of the end group. This cross section is nearly always completely determined when the end group is given, hence we may take the areas at the foot of the curves as being the maximum cross sections of the end groups of the molecules, under the packing conditions in the film.

In curves II to VI, it is found that mere compression of the films forces the molecules eventually into the state of close-packed chains. It is necessary to account for the possibility of diminishing the area by compression, as at no compression we have supposed that the cross section of the head (end-group) is large enough to prevent close packing. Either the heads of the molecules are compressible, or they can be tucked away into recesses in the neighbouring chains, probably by a slight movement up or down of neighboring molecules. It seems likely that the second alternative is the correct explanation. The rearrangement by forcing the heads into recesses in neighbouring chains could not occur if the end groups were too long vertically to fit into the recesses; and if the zigzags in the hydrocarbon chain are formed with the bonds between successive carbon atoms set at the tetrahedral angle of stereochemistry, it would not be possible to accommodate bulges on the molecules, which extended over more than two carbon atoms distance vertically, into the adjacent chains and still allow the chains to be closely packed. Curve VII shows that the benzene ring cannot be tucked away. Actually the compressibility of this curve, calculated in diminution of volume for a given increase in lateral pressure, assuming that the thickness of the part of the film which resists the lateral pressure, is 6 Å.u. or approximately the length of the benzene ring, is of the same order of magnitude as the compressibility of benzene; this is corroborative evidence that the resistance to compression is wholly provided in the case of these benzene derivatives by the close packed benzene rings, and it shows that the heads of the molecules are not more compressible in the films than are the same atomic groupings in matter in bulk. Another head which cannot be compressed in films is the urea group, $\text{NH}\cdot\text{CO}\cdot\text{NH}_2$, curve VIII.

Thus two main states of the condensed films can be distinguished, that of close-packed chains, and that of close-packed heads. The films with close-packed heads can be further subdivided into those which can undergo considerable re-arrangement by compression, probably by a vertical rearrangement of the molecules, the widest portions packing into recesses in the chains,

and sometimes being wholly accommodated in these, so that the chains can pack closely; and those where the heads of the molecules form of themselves a stable structure which cannot be compressed laterally (except to the extent that all matter can be compressed in bulk) into any other packing. The compressibility of curve I, the close packed chains, is of the same order of magnitude as that of liquid paraffins in bulk, if one takes the thickness of the film resisting the compression as the length of the molecules, the length being estimated on the assumption that the density of packing in the film is the same as in the substance in bulk.

The results on surface films are too frequently quoted as if they gave direct information as to the length of the molecules. The actual measurements are of cross section, and only the order of magnitude of the length can be obtained, by an assumption as to the density of the films; but that the density in the condensed films is of the same order of magnitude as that in the substances in bulk is clear, since the area per molecule calculated on the assumptions that the volume of a CH_2 group in the films is 29.4 cub. Å.u., the same as in organic compounds at their melting points, and that the vertical height of this group in the films is 1.5 Å.u., or the distance apart of the centres of carbon atoms in the diamond, is 19.6 sq. Å.u., the observed area of curve I, being 20.4 sq. Å.u.

The principal areas of groups as packed in the films, are

	sq. Å. u.
Hydrocarbon chains.....	20.4
- $\text{CH}_2\text{CH}_2\text{COOH}$	25.1
- CH_2OH	21.7
- $\text{CH}_2\text{CH}_2\text{COOR}^*$	22
- $\text{CH}=\text{CHCOOH}$	28.7
- $\text{CH}=\text{CHCOOR}$	28.7
- CONH_2	less than 21
-CN.....	27.5
- $\text{C}_6\text{H}_4-\text{OH}$, - OCH_3 , or - NH_2	23.8
- $\text{C}_6\text{H}_4\text{NHCOCH}_3$	28.2 or 25.8
- NHCONH_2	26
- NHCOCH_3	24.2
- $\text{CHBr}-\text{COOH}$	26 to 32
- $\text{CH}_2\text{CH}=\text{NOH}$	25

Hydrolecithin.....	52
Cholesterol.....	39
Triglycerides.....	63
Glycol dipalmitate.....	42

* R may be methyl, ethyl, or allyl.

SHAPE OF THE MOLECULES

It is evident that the length of the molecules must be much greater than the thickness; assuming the density in the films to be the same as in bulk, the length of the molecule of stearic acid, or the thickness of the condensed film, is 26 Å.u.; the square root of the cross section is only 4.5 Å.u. Bulky groups such as bromine increase the cross section of the head of the molecule; even a double bond, as in the iso-oleic acid which has a double bond in the α - β position, greatly increases the area of packing. When however the double bond is in the middle of the chain, in elaidic and erucic acids, there is no enlargement of the cross section. The double bond may be a kink in the molecule, rather than a local swelling. These measurements are exactly what would be expected from the organic structural formulae, proving that it is legitimate to regard these formulae as models, at any rate approximately accurate, of the molecules.

CHANGES OF STATE IN THE CONDENSED FILMS. ALLOTROPY

Figure 2 gives two curves for the ureas, $RNHCONH_2$. The curve I is obtained above a transition temperature; it shows that the chains are closely packed. The curve VIII is obtained below this temperature. The transition was been observed on the C_{16} , C_{17} , C_{18} , and C_{20} ureas (14, 18), the only effect of increasing the length of the chain being to raise the transition temperature slightly. The low temperature curve is that of close packed heads, area 26 at no compression; the film is solid, and compression does not re-arrange the molecules. It appears that below the transition temperature the packing of the heads alone determines the area and gives strength to the film. The transition temperature is therefore a melting of the structure formed by

the heads of the molecules; when this is destroyed, it is possible to pack the molecules closer together, till the chains are in contact. Increase of pressure lowers the transition temperature slightly, as it should, since the high temperature packing has a smaller area than the low. Near the transition temperature, if the pressure is raised so that the close packed chains becomes the stable formation, the change from the close packed heads to this is not instantaneous but takes some minutes; and the expansion to the state of close packed heads on reducing the pressure in this region may also be delayed. There is therefore a possibility of much hysteresis in the films (18).

The same kind of change between two allotropic forms of the films occurs with hexadecyl acetanilide $R\text{—}\langle\bigcirc\rangle\text{—NHCOCH}_3$ (18). Here the low temperature packing has the greater area; but the high temperature packing is not that of close packed chains, nor even that of close packed benzene rings, but has a larger area than either.

The acetamides, $R\text{NHCOCH}_3$, show a different change, the low temperature film being solid, and having the close packed chain formation; the high temperature film is liquid and has a larger area. It may be that below the transition temperature or melting point, which is in this case a sharply marked point, there is a cohesion which keeps the heads of the molecules tucked away in the chains; at a certain temperature this breaks down, and the molecules begin to rotate about a vertical axis, at once destroying the solid structure of the film and sweeping out an area greater than that of the closest packing.

SPONTANEOUS CHANGES IN AREA OF THE FILMS

With condensed films, the pressure equilibrium is not always attained instantaneously. This is sometimes due to slowness of the molecules in attaining their final packing, as in the case of the urea derivatives near the transition temperature. Hydrolecithin seems to be another such case; at room temperature this film, if suddenly compressed, takes some minutes to reach the final area, the pressure falling in the interval; if suddenly

allowed more space on the surface, after being compressed, the pressure falls below the equilibrium value and then rises. The complicated constitution of the molecule may account for its slowness in settling down to the most stable packing (21). In other cases, it is possible that a slow change in the film is due to chemical reaction altering the size of a part of the molecule; the amine hydrochlorides on distilled water form films which increase slowly in area for about ten minutes. In other cases, a change in area may be due to an easily recognizable chemical change in the film; the lactone of γ hydroxystearic acid is hydrolyzed on a normal aqueous solution of caustic soda to the acid; the lactone is a condensed film, and the hydroxyacid, on this solution, a very strongly expanded film, so that the change can be followed by the rate of increase of pressure or area. These reactions of the films need further investigation, which has been delayed until the main facts of the structure could be ascertained.

INFLUENCE OF TEMPERATURE

Condensed films show little if any change of area with temperature. As the temperature is raised, and the thermal agitations of the molecules made more intense, the structure of the condensed films may break down in one of two ways. The principal forces stabilising the condensed films are the lateral attractions between the hydrocarbon chains, and the vertical attraction of the polar heads to the water. In some cases, as with the long chain phenol methyl ethers (16), rise of temperature causes the anchorage to the water to break down first; then the films collapse spontaneously to form masses on the surface.⁷

EXPANDED FILMS

In nearly all cases, however, it is the lateral adhesion which is overcome before the vertical. At a certain temperature the

⁷ Cary and Rideal (27) bring evidence to show that many of the films which can be examined at leisure, once spread by the aid of a solvent, are thermodynamically unstable. The action of heat in causing the collapse is therefore probably one of accelerating the attainment of equilibrium.

area begins to increase, then increases rapidly with further rise, until about ten degrees above the temperature of first increase of area the rate of expansion with rising temperature drops abruptly to an amount about the same as the coefficient of thermal expansion of a gas. This expansion was first recorded by Labrouste (29), and has been studied mainly by the author. Figure 3 shows the F - A curves for myristic acid on dilute HCl, at various temperatures from 2.5° up to 34° . The curve at 2.5° is the condensed curve. That at 34° is the fully expanded curve; further rise of temperature merely moves it nearly parallel to

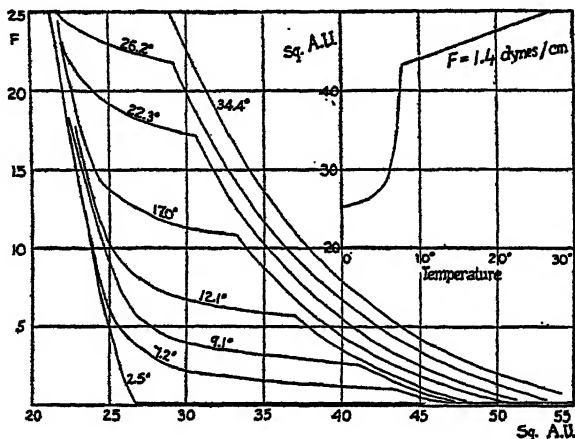


FIG. 3. EXPANSION OF MYRISTIC ACID

itself to the right and upwards. The intermediate curves show that there is always a well defined point at which pressure causes the expanded film to begin to condense; this point is found at a higher pressure, the higher the temperature. The effect of pressure after the condensation has commenced is not exactly that of a pressure on a change of state, for the transition portions of the curves between the condensed and expanded states are not horizontal. In fact at the condensed end of the transition curve there is only a very gradual change of area.

There are two classes of expanded film, those which pass into the gaseous film with a definite discontinuity and show a con-

stant vapor pressure region, and those which show no discontinuity or surface vapor pressure; cases of the first are the higher fatty acids (above C_{13}) as seen in figure 1, the bromo-acids, the unsaturated acids oleic, elaidic, and erucic, and probably palmitic nitrile. The fatty acids such as lauric, which is seen from figure 1 (or better from 19, fig. 1) to be above the "critical evaporation temperature," and the ethyl esters, do not show this constant vapor pressure, and the curves pass continuously into the gaseous curves.

The first class of expanded films tend to a definite area at no compression, which can be fairly accurately estimated by extrapolation, since the expanded curves continue down to the commencement of the vapor pressure, which is not greater than a few tenths of a dyne. It is a curious fact that the area of these expanded films at no compression is always, when one chain is present in the molecule, about 50 sq. Å.u. per molecule. There is evidently much cohesion in the films in this condition, but the structure is not yet clear. The area is much too great for the molecules to be vertical and closely packed; it is also much too small for the molecules to be lying flat. It is possible that the long chains are coiled down in a helical form, with vertical axis; alternatively the chains may be oscillating through a variety of orientations, passing through horizontal and vertical positions. To account fully for the complicated phenomena shown in the curves of figure 3 seems no easy task; it is however an important one, since the expanded state of the films is very common.

The esters belong to the second class of expanded films, and reach larger areas (about 60 or 70 Å.u. at 1.4 dynes per centimeter) than the preceding; there is no discontinuity between them and the gaseous state. The F - A isothermals of the esters (19, fig. 2) show something approaching a discontinuity for ethyl palmitate, though there is no horizontal vapor pressure region; as the chain is shortened there is no sign of a discontinuity, but the curves pass smoothly into the gaseous condition, from areas below 100 sq. Å.u. It appears that the same kind of forces are operating, in the case of those ethyl esters not far removed from their expansion temperature, which cause

the expanded films of the first class to have a definite limit on their expansion; there is in the case of the esters, some peculiarity which prevents the cohesion being as strong as with the fatty acids. Further work is being done on these expanded films, but the solution does not seem close at hand yet.

A regularity has been established connecting the temperature of expansion from the condensed to the expanded films, with the length of the chain. The temperature of expansion rises by about 10° near 0° , and about 7° near 60° , when one carbon atom is added to the chain. This has been found with every one of ten homologous series, although the absolute temperature of expansion depends greatly both on the chemical nature of the end group, and the composition of the solution. This influence of the length of the chain shows that it is the lateral attraction between the parallel, close packed molecules in the condensed state, which is being overcome in the change to the expanded state; this attraction is naturally longer, the greater the length of the hydrocarbon chains.

INFLUENCE OF CHOLESTEROL

Leathes (30) found that cholesterol, mixed with myristic or other fatty acids in the films, has the property of reducing the area of the acid in the film, from the expanded, to the condensed value. It has been found (21) that very few other substances have this property; tripalmitin and one or two other very bulky molecules have some condensing action, though not to the same extent as cholesterol.

INFLUENCE OF PERMANGANATE IN THE WATER

Potassium permanganate in the water on which the films are formed has practically no effect on most films with a saturated chain; but it changes a condensed, or expanded film, of an unsaturated substance with a double bond in the middle of the chain, into a gaseous film, increasing enormously the pressure at a given area. Doubtless this is due to the attraction of the of the permanganate on the middle of the chain assisting the molecule to lie flat, thus causing it to assume the characteristic

orientation of the gaseous film, and diminishing the opportunity for the action of the lateral cohesion between the molecules.

EQUILIBRIUM BETWEEN CRYSTALS OR DROPS, AND THE FILMS

Cary and Rideal (26-28) have studied the equilibrium between long chain substances in bulk on the surface, and the monomolecular films, and also the rate of spreading from crystals to form films. There is a definite maximum surface pressure in the film which stops further spreading the equilibrium pressure; the molecules leave the crystal at a rate which is at any temperature proportional to the difference between the equilibrium pressure and the actual pressure at any moment in the film, and also to the perimeter of the crystal. The rate of spreading increases rapidly with the temperature; the equilibrium pressure increases also with the temperature, the rate of this increase being linear. In the curves relating equilibrium pressure to temperature, there are some curious discontinuities, the meaning of which is not yet clear. The measurements were of the surface tension of the contaminated surfaces, by observing the force needed to withdraw a ring; as the substances were insoluble, the direct measurement of surface pressure would have been applicable and perhaps more convenient. Adam and Jessop (20) continued the observations down to very low pressures on myristic acid, obtaining complete agreement with the above theory of the mechanism.

Hardy (31), Wilson Taylor (32), and others, have shown that when a liquid spreads on a water surface, it is first drawn out into a more or less uniform thin sheet, which then undergoes disruption until it consists of a thin, almost certainly monomolecular, film, in equilibrium with numerous drops of liquid. The author regards the mechanism of this spreading, as due to the action of the tangential components of the thermal agitation of the water molecules at the surface, dragging out the oil molecules rapidly along the surface. Owing to the viscosity of the oil, the lowest layer of oil molecules drags the remainder of the drop out along the surface; when the lowest layer is spread over the whole surface and becomes quiet, the upper

layer adjusts itself as rapidly as possible to the position of equilibrium, which is of course that of large drops in equilibrium with a mono-molecular film. This phenomenon is sometimes quoted as due to a squeezing action, the attraction exerted by the water on layers of the oil remote from the water squeezing out the lowest layer along the surface; and hence as proof that the attraction of the water extends to several molecules distance into the oil; but if the thermal motions of the water are taken into account, the phenomenon does not require that the molecular attractions should extend over more than infinitesimal distances.

THE OPTICAL PROPERTIES OF SURFACE FILMS

A surface film makes an appreciable difference to the reflection of light at a solid or liquid surface. Fresnel's law of reflection requires that if the transition between air and a material of refractive index n is sudden, the light reflected when the angle of incidence is the Brewsterian angle ($\tan^{-1} n$) will be completely plane polarized, and that light polarized perpendicularly to the plane of incidence will not be reflected at all at this angle. Jamin and others found that as a rule the polarization was not perfect, the residual light being elliptically polarized, from liquid surfaces. Drude found that Fresnel's laws were often obeyed by freshly cleaved surfaces of crystals, but if these were left in the air for a time, elliptical polarization of the reflected light was the rule. Rayleigh (6) cleaned the water surface carefully, and found that it then reflected as if the transition was abrupt; a film of olive oil, only one fifth of the density needed to stop the movement of camphor on the surface, made an obvious difference, causing ellipticity. He did not succeed in tracing a definite relation between the degree of ellipticity of polarization of the reflected light, and the surface pressure.

Raman and Ramdas (33) have recently found that the surface scattering of light from a water surface, which is appreciable in the case of clean water, may be increased 100 per cent by a film of oleic acid sufficiently dense to stop movements of camphor. Less dense films had no perceptible effect, so that it appears

that an amount which causes noticeable deviation from Frensel's laws does not necessarily affect the surface scattering of light.

FILMS ON MERCURY

The difficulties in obtaining a clean surface of mercury have so far prevented much information being obtained on the properties of the films which can undoubtedly form on this liquid. Optical methods have been employed by Fischer (34), Haak and Sissingh (35), Haak (36), and Reeser (37). Air appears to condense on the surface of mercury, in a film which affects the reflection of light; its thickness is estimated as of the order 20 Å.u., or several molecules thick. The proof that the mercury surface was really clean, when supposed to be so, does not appear complete, and possibly the result attributed to air is due to some other cause. Fischer gave the thickness of oil layers on mercury as less than 50 Å.u., and Reeser as of the order 20 Å.u.

Iredale (38) has investigated the variation of surface tension of mercury with the partial pressure of various organic vapors. This is equivalent to the investigation of the film at a liquid-air surface, by measuring the surface tensions of solutions of different concentrations, the vapor phase being of course the solution in which the concentration is varied. The course of the surface tension—partial pressure curves shows the films to be of the gaseous type, in the cases of methyl acetate and benzene. At partial pressures approaching saturation, the area per molecule becomes of the order 20 to 30 sq. Å.u.

A mere numerical coincidence, in order of magnitude, between the area per molecule in an adsorbed film, at the maximum observed adsorption, with the area of a condensed film, does not justify the conclusion that the film is condensed, for as Langmuir found, most gaseous films tend towards a minimum area of about 25 Å.u. at high compressions. Such conclusions have been drawn mistakenly. It is necessary to observe not only the area per molecule, but the whole course of the $F - A$ isothermals, before drawing conclusions as to the structure. Goard and Rideal's measurement (39) of the minimum area per mole-

cule, in the adsorbed layer of phenol on water was 23.8 sq. Å.u.; this is probably a merely accidental agreement with the author's measurement of the cross section of the tightly packed phenol groups in a condensed film (see table, p. 182). These absorbed films are gaseous and obey equation (3).

Burdon has recently described a method of obtaining a clean mercury surface, which may be very useful in the study of surface films. He has also obtained much interesting information on the spreading of various liquids on mercury (40).

THE EFFECT OF A SURFACE FILM ON THE EVAPORATION OF WATER

Rideal (41) has shown that surface films of fatty acids have a retarding effect on the evaporation of water. The retardation, though considerable in that the film offers a serious resistance to the passage of water molecules through the surface, is not easy to detect, as under ordinary conditions of evaporation what is measured as the "rate of evaporation" is merely the rate at which the vapor diffuses away from the layer of saturated vapor near the surface. By evacuating the space over the water until it was on the point of boiling, rates of evaporation up to 0.4 per cent of the theoretical were obtained; and these were slowed down by 20 to 50 per cent if either a condensed or an expanded film was present. Increasing the surface pressure increased the retardation of evaporation. Evidently a film very greatly retards the real rate of the molecules leaving the surface; it will be difficult to make quantitative measurements until the retarding effect of the slow diffusion away from the surface can be still further eliminated.

CATAPHORESIS OF SURFACE FILMS

Some recent experiments (21) have failed to detect any force on a film on water, caused by applying a fall of potential of about 70 volts per centimeter along a water surface. The film was about 130 sq. cm. in area, on water in a trough 4 mm. deep, made of ebonite, and a force of 0.005 dyne per centimeter could have

been detected. Films of fatty acids and of amine hydrochlorides were used, and at present no explanation can be given of the negative result.

ADSORPTION OF WATER ON SALT SOLUTIONS.

If the surface tension increases with increasing concentration, then there is less of the solute in the surface layers than in the bulk of the solution. The amount of the deficiency of solute in the surface, or "negative adsorption" may be calculated by Gibbs' equation, if the surface tensions and activities of a series of different concentrations of solution are known. Interesting results have been obtained on the assumption that the excess of water at the surface is present in the form of a thin layer of pure water spread on the top of the solution, which is supposed to remain of the same concentration as in the interior, right up to the boundary of this water layer. If $-\Gamma$ be the deficiency in the solute per square centimeter of the surface, calculated from Gibbs' equation, τ the thickness of the superficial layer of water, m the molality of the solution (mols per 1000 grams), then

$$\tau = - \frac{1000\Gamma}{m}.$$

Langmuir (11) calculated the thickness of the adsorbed water layer in this way, using the approximate form of Gibbs' equation, finding values of τ from 3.3 to 4.2 Å.u. The length of the side of a water molecule, assuming it to be a cube and that these cubes are close packed in liquid water, is 3.1 Å.u., so that these figures lend some support to the view that the surface of the salt solutions which show negative adsorption are covered by a layer of water without salt, 1 molecule thick.

More recent work by Goard (42) and Harkins and others (43, 44) shows that in all cases the thickness τ of the water layer, calculated on these assumptions, diminishes with increasing concentration of the solution; from about 5 Å.u. at low concentrations to about 2 Å.u. in strong solution. The most probable explanation of this variation is that the surface of the solution is not quite free from salt, in the stronger solutions at any rate; there is a tendency to form a layer of pure water at the surface, but the diffusion pressure of the solute in the stronger solutions

tends to force some salt molecules to the surface. The forces at the surface of a liquid are an inward attraction exerted on those molecules which are in the surface layer; owing to the residual affinity of the salt being higher than that of the water, these forces are strongest on the salt molecules and therefore the surface is richer in water than the rest of the solution; unless however the difference in residual affinity between the water and the salt is very great, the tendency of the diffusion forces to keep the concentration uniform is not entirely masked even in the surface layer. The fact that the amount of adsorbed water is so nearly equal to a monomolecular layer of pure water spread on the surface is evidence of the very small range over which the surface attractive forces are operative, under ordinary conditions.

THE NATURE OF THE ADSORBED FILMS IN STRONG SOLUTIONS OF CAPILLARY ACTIVE SUBSTANCES

Schofield and Rideal (22) find that both with ethyl alcohol and water at a liquid-air interface, and with pyridine and water, at a water-mercury interface, the adsorption calculated from the exact form of Gibbs' equation rises from zero to a maximum as the concentration of the more strongly adsorbed component increased, and then falls off. In each case the maximum occurs at a mol fraction of about 0.3 and an area per molecule of 24 Å.u.

The apparent adsorption then *decreases* fairly rapidly with increasing concentration, falling to less than half of its maximum value at a mol fraction of 0.7. The maximum adsorption is a layer practically closely packed, of the alcohol and pyridine; it is unlikely that this layer will be disturbed by further increase in the concentration, and therefore it is undesirable to attempt to explain the apparent falling off in adsorption by assuming the adsorbed layer to become less dense. But if as the alcohol or pyridine concentration increases, there is a tendency to commence the formation of a second layer of *water*, adsorbed underneath the outermost layer of alcohol or pyridine, the net effect on the surface layer will be to diminish Γ . The formation of a double adsorbed layer, consisting of the most easily adsorbed constituent outside, and the less easily adsorbed one immediately

underneath it, appears the best explanation possible at present of this apparent falling off in adsorption in strong solutions.

THE METHODS OF INVESTIGATION OF INSOLUBLE SURFACE FILMS

For full details the reader must refer to the original papers, especially (14, 19). Some points are important as affecting the design of any instrument to be used with the films, and may be mentioned here.

Reliable methods of cleaning the surface are essential, as well as of frequently ascertaining its cleanliness. Far the best method is to use heavy barriers working on a trough filled to the brim with the liquid, the top of the sides being about 1 cm. wide. The barriers must be coated with paraffin wax, and the top of the sides of the trough also. Ordinary high melting commercial wax is quite satisfactory. Its function is to prevent the water spreading over the trough, and so escaping under the barriers outside the trough and carrying the films with it; the wax does not cause appreciable contamination of the water surfaces, up to about 30°, and approximate measurements can be made above the melting point of the wax.

With these barriers, tests of the cleanliness can be made simply by reducing the area of the surface and noting whether any surface pressure is developed. The films being invisible, frequent tests must be made.

For some purposes an adequate method of cleaning a water surface is to have a vessel of any shape, with a level brim and a means of introducing water at the bottom; an ordinary funnel serves well. The water is allowed to run in from the bottom for a minute or two, and any contamination is swept over the top, the surface remaining clean for a long time after stopping the flow. This method does not require any special cleanliness in the vessel.

To measure the surface pressure, it is necessary to have a float freely moving in the surface, which nevertheless does not allow the film to leak past the ends; and to apply a measured force to this float. Various devices have been used to prevent leaks

past the ends of the float. Langmuir and the author have used a float about 1 mm. from the sides of the trough at each end, and blocked the gaps by blasts of air from jets directed at a suitable angle on the surface of the water. These unfortunately disturb the force on the float to some extent, the error possibly reaching 0.5 dyne per centimeter when blasts strong enough to prevent leaks under high pressures are used. With this arrangement, a simple balance on the bell-crank principle has been used for measuring the forces.

A more satisfactory method, used by the author and Jessop (19), is to end the float about 1 cm. away from the sides of the trough, and block the gaps with fine gold ribbon, bent into an S shape, placed vertically in the water surface, and soldered to the ends of the float and to blocks pressed tightly against the sides of the trough. This arrangement allows of measurement down to 0.01 dyne, and is leak proof up to the highest pressures. Combined with an optical lever to record the position of the float, and a torsion wire system to measure the forces (19) it is probably the most satisfactory method available now.

Marcelin (45) describes two pieces of apparatus; a sensitive one with a vertical torsion wire, but without any means of blocking the gaps at the ends of the float. The author has shown (46) that the results obtained with this are probably seriously in error. The other instrument is on the principle of a two dimensional aneroid; it consists of a frame in the surface, one side of which is a thin, flexible piece of mica, attached to a sensitive lever system. The water in the interior of the frame is supposed to be clean, and the pressure of the film outside moves the mica and the lever system. There is however no means provided of cleaning the surface inside of the frame.

The experiments are not difficult, with proper arrangements, but a constant watch must be kept for leaks, and accidental contamination, or inexplicable results are liable to occur.

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- (45) MARCELIN: Ann. phys., 4, 459 (1925).
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A REVIEW OF PHOTOCHEMISTRY

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Photochemistry is one branch of the study of the effects of radiations on chemical reactions. Studies of the effects of various types of radiation have received a great deal of attention and in recent years a comprehensive theory of the chemical effects of alpha and beta particles has permitted a general correlation of most of the important data.¹ Unfortunately, while Photochemistry may be said to be much older than its fellows, it is at present in a far more unsatisfactory state.

The Radiation Hypothesis of Chemical Action² has brought the subject of Photochemistry into prominence during the past few years. It is not the purpose of this paper to discuss the Radiation Hypothesis, but we would like to show something of the developments in the field of Photochemistry and to present some of the hypotheses advanced to interpret the data obtained. The subject is so vast and its ramifications so numerous that it is necessary to limit the field very carefully. For this reason no mention will be made of studies relative to photographic processes and little attention will be given to the hydrogen-chlorine reaction. This reaction has probably received more attention

¹ See Lind, *The Chemical Effects of Alpha Particles and Electrons*, The Chemical Catalog Company, 1921 and for more recent work a series of articles by Lind and his coworkers in the *Journal of the American Chemical Society*.

² Trautz, "Sitzungsber. Heidelberger Akad. Wissenschaft," 1917; Lewis, *Trans. Faraday Soc.*, 17, 573 (1922) (This article presents a brief statement of Lewis' viewpoint. Many other articles have been published, principally in the *Journal of the Chemical Society (London)*); Perrin, *Annales de Physique*, 11, 5 (1919). Many other authors have discussed the Radiation Hypothesis critically. Perhaps the most interesting and significant recent articles on the subject are by Tolman, *J. Amer. Chem. Soc.*, 42, 2506 (1920); 45, 2285 (1923); 47, 1524 (1925); 47, 2652 (1925) and by Lewis and Smith, *ibid.*, 47, 1508 (1925). Harned (*Journ. Franklin Institute*, 196, 181 (1923)) has given an excellent review of the subject.

than any other single photochemical reaction. Nevertheless this reaction, which seemed to be relatively simple at the time the first studies were made, has proved to be very complex. A great deal has been learned, but there are still many points of disagreement among the various workers. Marshall³ has given a very complete and satisfactory review of the work on this particular reaction.

There are many reasons why the subject of Photochemistry is in such an unsatisfactory state. In general, data on the kinetics of reactions are very difficult of interpretation. It is only recently that the effects of even minute traces of impurities have been recognized. Perhaps the work of Baker⁴ on extremely dry liquids has served to bring this matter to general attention more than any other single piece of work. It would seem that many chemical reactions take place only in the presence of some catalyst, i.e., either the walls of the vessel or traces of moisture or some small amount of foreign substance whose existence is frequently ignored. The most notable exceptions seem to be the decomposition of nitrogen pentoxide which has been studied by Daniels and his coworkers⁵ and the decomposition of sulfuryl chloride which has been studied by Smith.⁶ The decomposition of nitrogen pentoxide has been found to be a monomolecular reaction and the specific reaction rate is found to be constant over a wide range of concentrations. By far the great majority of reactions do not behave in such a simple fashion. Many authors have arrived at conclusions with regard to the mechanisms of certain reactions by means of studies of chemical kinetics. It would seem advisable to regard most of these conclusions with a great deal of skepticism. It is always advisable to present some picture of the mechanism of a reaction if it is possible, since the human mind is so con-

³ Marshall, *Trans. Amer. Electrochem. Soc.*, Preprint for meeting held April 22, 1926.

⁴ Baker, *Phil. Trans.*, 79A, 583 (1888); *J. Chem. Soc.*, 65, 611 (1893); 81, 400 (1902); 91, 1862 (1907), 101, 2339 (1912); 121, 568 (1922).

⁵ Daniels and Johnston, *J. Amer. Chem. Soc.*, 43, 53 (1921); 47, 1602 (1925); Hirst has obtained results agreeing with those of Daniels, *J. Chem. Soc.*, 127, 657 (1925).

⁶ Smith, *J. Amer. Chem. Soc.*, 47, 1862 (1925).

stituted that it must have a picture as a working basis. The formation of pictures serves a useful purpose in stimulating research and the criticism and consequent rearrangement of these pictures will continue to lead to many valuable conclusions. Far too frequently, however, authors have been lead to form pictures and then to seek an interpretation of all data obtained subsequently in terms of these pictures, thereby overlooking many important points. It must be borne in mind that several different pictures will usually interpret a given set of data with equal exactness.

In general the application of thermodynamics to photochemical reactions has reached only a very elementary stage. Einstein⁷ has derived the law of Photochemical Equivalence and the quantum theory has given a means of applying energy relationships to photochemical reactions. This subject has been well treated in other reviews.⁸ Most of the applications of quantum theory to photochemical reactions have been based on postulations of "activated" states. The exact nature of the activated state is usually unknown, but it is assumed that the difference in energy between the activated molecule and the normal molecule can be calculated from the wave length of light absorbed.⁹ However it would seem that the postulation of a single definite activated state and the calculation of the energy of activation is not justified in many cases. As an example we may cite the photochemical decomposition of solutions of oxalic acid. Oxalic acid presents an absorption band¹⁰ which apparently begins between 3000 and 3500 Å and it is well known that solutions of oxalic acid are decomposed by the ultra-violet radiation in sunlight¹¹ and that the amount of decomposition is a function of the quantity of energy absorbed. If it is assumed that a certain "activated state" is necessary as a preliminary to the decomposition of oxalic acid

⁷ Einstein, *Ann. Physik*, **37**, 832 (1912).

⁸ Harned, *loc. cit.* ref. 2; Dushman, *A Treatise of Physical Chemistry*, edited by H. S. Taylor, D. Van Nostrand Company, 1924.

⁹ See Tolman, *J. Amer. Chem. Soc.*, **42**, 2506 (1920) for a discussion of this matter.

¹⁰ Hantzsch and Scharf, *Ber.*, **46**, 3570 (1913).

¹¹ See, for example, Bongiovanni, *Gazz. chim. ital.*, **46**, 127 (1916).

and that the difference in energy between the normal molecule and the activated molecule can be calculated from the position of the absorption band, then we are apparently forced to conclude that the activated state of oxalic acid in the presence of uranyl salts is different from the activated state in solutions of pure oxalic acid, for solutions of oxalic acid containing uranyl salts are decomposed by visible light to a slight extent.¹² There is, of course, the possibility that the activated state in both cases is the same and that absorption of light by the uranyl sulfate may cause a subsequent activation (by collisions of the second kind) of the oxalic acid molecules which have an energy content greater than the average. The uranyl sulfate would merely act as a holder of the energy until an oxalic acid molecule of the proper energy content was able to absorb it. In many cases it would seem doubtful whether an energy of activation can be calculated directly from the wave length of the absorption band. For many reactions the number of molecules reacting per quantum absorbed is a decided function of the wave length and it would seem that in many cases the amount of substance reacting is proportional to the amount of energy absorbed.¹³ This is in contradiction to Einstein's law, which would tell us that every quantum absorbed should have the same effect as any other quantum and that therefore the amount of substance reacting per calorie of energy absorbed should be greater at the longer wave lengths. It may be answered with reason that the conditions postulated by Einstein probably have not been fulfilled for any photochemical reaction studied and that Einstein's law should be applied only to the primary process, presumably the process of activation. Thus as the next logical step we would find it necessary to postulate a series of activated states, or perhaps a gradual variation in the energy content of the absorbing molecules, in such a way that the probability of reaction divided by the probability

¹² Mathews and Dewey, *Jour. Phys. Chem.*, **17**, 211 (1913); Landau, *Compt. rend.*, **156**, 1894 (1913); Anderson and Robinson, *Jour. Amer. Chem. Soc.*, **47**, 718 (1925).

¹³ See, for example, Bowen, *Phil. Mag.*, **50**, 879 (1925); *Trans. Far. Soc.*, 1926 (advanced proof).

of return to the unactivated state without reaction would be a function of the energy absorbed and would be greater for the shorter wave lengths. If this is the case one should expect a certain amount of fluorescence and consequent light scattering for the longer wave lengths absorbed, or at least an increase in temperature of the system, which fluorescence and increase in temperature would be less noticeable as one proceeded to the shorter wave lengths. Certain reactions have been studied and data obtained which might be said to agree with such assumptions, but we may safely say that statements of this sort rest on doubtful experimental evidence.

There have been many attempts to classify photochemical reactions. The classification proposed by Bodenstein¹⁴ seems to be quite logical. His classification is based on the Einstein law of photochemical equivalence. He classifies the reactions into primary and secondary, depending on whether the reactions obey Einstein's law or not. For primary photochemical reactions the number of molecules reacting per quantum is either unity or some small whole number, within the experimental error. As we have mentioned above, probably no reaction studied has been carried out under the conditions postulated by Einstein. For primary photochemical reactions, therefore, we may consider that each quantum absorbed leads to an activated molecule and that the probability of reaction after activation is one. If any other small whole number of molecules is found to react per quantum absorbed, it is possible to postulate collisions between activated products and unactivated molecules or between activated molecules and unactivated molecules in such a way that energy is transferred or that both molecules entering into collision react. Measurements of light intensity are difficult to carry out and in particular is it difficult to get an exact measure of the amount of light absorbed in a photochemical process, making due allowance for scattering and for absorption by the walls of the container. In addition it must be remembered that we are dealing with large numbers of molecules and that at best any

¹⁴ Bodenstein, *Z. physik. Chem.*, **85**, 333 (1913).

data obtained will be of a statistical character. Thus if the probability of reaction after activation were not unity, but when a molecule did react a chain were started involving activation by the products, it would be possible to find an agreement with Einstein's law. In this same manner it would be possible to explain a deviation from the law, while an agreement might be fortuitous. In fact there seems to be some evidence that an activated molecule will not decompose or react spontaneously but that it will only do so upon collision with a second molecule. This would make a reaction which apparently behaves as a reaction of the first order really a second order reaction. Some evidence has been obtained on this point by studying certain gas reactions at pressures so low that the duration of the activated state is of the same order of magnitude as the mean time between collisions. Under such conditions the order of the reaction should be higher than at higher pressures. The decomposition of nitrogen pentoxide in the dark apparently does not deviate from a first order equation even at very low pressures.¹⁵

Secondary photochemical reactions are those which exhibit a wide deviation from Einstein's law, many molecules reacting per quantum absorbed. Here again we must bear in mind that we are dealing with final reactions and not with the activating process. It would seem necessary to postulate chain mechanisms of some sort to account for reactions of this type.

As would be expected, those reactions which proceed with a large decrease in free energy belong in the class of secondary photochemical reactions, while those reactions which proceed with a slight negative free energy or a positive free energy fall in the class of primary light reactions. However this generalization can only be made when conditions are such that a deviation from Einstein's law is permitted. Marshall¹⁶ has shown that monatomic hydrogen is capable of initiating the reaction between hydrogen and chlorine and that the yield per hydrogen atom is a function of the pressure, being greater at higher pressures. He has also investigated the photochemical reaction between hydro-

¹⁵ Daniels, *J. Amer. Chem. Soc.*, **47**, 1602 (1925).

¹⁶ Marshall, *J. Phys. Chem.*, **29**, 842 (1925).

gen and chlorine at low pressures¹⁷ and has found that the yield of hydrogen chloride molecules per quantum is twenty at a pressure of 0.001 cm. and increases to over 25000 at a pressure of 6.0 cm. Thus while we have given the rule that those reactions which involve a large decrease in free energy would probably be secondary photochemical reactions, we must qualify our statement and add that conditions must be such that some sort of chain mechanism can take place. We can now explain the effect of inhibitors as being due to action in stopping the chain, while the effect of the reduced pressure would be to decrease the probability of collision between an activated and an unactivated molecule during the duration of the activation.

Other classifications of photochemical reactions have been proposed. Schidlof¹⁸ has put all photochemical reactions into three groups as follows: (1) Exoenergetic reactions. An increase in frequency displaces the equilibrium in the same direction as an increase in temperature for exothermic reactions. (2) Endoenergetic reactions. The effect of change in frequency is the reverse of the above. (3) Reactions for which energy is neither taken up or given out. For these reactions the equilibrium would be independent of the frequency and radiation may act simply as a catalyst. Unfortunately, while this classification obviously has much in common with the classification of Bodenstein, very few measurements have been made of the effect of radiation on equilibria. There is no doubt that such an effect does exist as evidenced by the fact that reactions may be made to proceed in both directions by a proper choice of wave length and of other conditions. For example, Warburg has found that maleic acid may be converted into fumaric and vice versa.¹⁹ The rate of change of fumaric into maleic is more rapid than from maleic into fumaric and the effects of various wave lengths are not identical.

Plotnikov²⁰ has classified photochemical reactions according to

¹⁷ Marshall, *ibid.*, 29, 1453 (1925).

¹⁸ Schidlof, *Arch. sci. phys. nat.*, 37, 493 (1914).

¹⁹ Warburg, *Sitzb. kgl. preuss. Akad.*, 1919, 960.

²⁰ Plotnikov, *Z. physik. Chem.*, 73, 573 (1911).

their temperature coefficients. He has given three classifications for which the factors for change in reaction rate with a ten degree rise in temperature are 1.04 ± 0.03 , 1.20 ± 0.03 and 1.39 ± 0.03 . Plotnikov's original idea seemed to be that the electron configurations of the reacting substances determined the nature of the photochemical effect and therefore the temperature coefficient of their photochemical reactions. Thus he found that the temperature coefficients of several reactions involving the addition of iodine were the same as for several reactions involving the addition of bromine, i.e., an increase in reaction rate of about 1.4 times for a ten degree rise in temperature. Tolman²¹ has considered the problem of the temperature coefficients of photochemical reactions in detail. He concludes that when the temperature coefficient is unity that the average energy of the molecules which enter into reaction is practically the same as the average energy of all the molecules. This means that the average molecule has as good a chance of picking up a quantum and reacting as any molecule in a quantum state far removed from the average. It is thus easy to interpret the temperature coefficient which Plotnikov has found for one class of reactions. With regard to the other classes Tolman finds that there is no theoretical reason why the temperature coefficient should have any particular value other than unity. If the temperature coefficient is not unity, the chance that a given molecule will be able to pick up a quantum and react will depend on the particular quantum state in which it finds itself. Tolman suggests a possible effect of the decrease in viscosity of water which might account for the temperature coefficient 1.20.

We will divide the following discussion into several parts. The first part will consist of a study of photochemical reactions from the standpoint of their active wave lengths, with a presentation of some of the ideas relative to active states and the application of thermodynamics. It would be obviously impossible and equally inadvisable to mention all of the photochemical reactions studied. The recent report of the Chemical Society²² has

²¹ Tolman, *J. Amer. Chem. Soc.*, **45**, 2285 (1923).

²² Allmand, *Annual Reports on the Progress of Chemistry*, **22**, 333 (1926).

covered the field of photochemistry from 1913 to 1925 in a very thorough manner. As a consequence, in part II we will limit ourselves to a few reactions which may be cited as examples.

PART I

Before starting on a discussion of photochemical reactions from the standpoint of wave length, we might mention possible methods of calculating the wave length which should be active for a given reaction. The simple equation of the Radiation Hypothesis

$$\frac{d \ln k}{dT} = \frac{N h \nu}{RT^2}$$

was proposed by Trautz, Lewis and Perrin.²³ The frequency of the active radiation is represented by ν , k is the specific reaction rate for the dark reaction, h is Planck's constant and N is Avogadro's number. This equation has been tested with great care by Daniels and Johnston²⁴ and has been discussed critically by various authors. Other authors have attempted to apply this equation²⁵ and we must admit that the evidence in favor of its applicability is rather unsatisfactory. For most reactions the temperature coefficient is such that a wave length in the near infra-red should be active. So far little work has been done on the chemical effects of the infra-red, but the results indicate that in very few if any cases does the infra-red exert any effect. Daniels²⁶ and Taylor²⁷ have studied the effect of infra-red radiation on the decomposition of nitrogen pentoxide and both have come to the conclusion that the infra-red is without any noticeable effect.

²³ Loc. cit., ref. 2.

²⁴ Daniels and Johnston, *J. Amer. Chem. Soc.*, **43**, 72 (1921).

²⁵ Taylor and Lewis, *ibid.*, **46**, 1606 (1924).

Capper and Marsh, *ibid.*, **47**, 2847 (1925).

Noyes and Kouperman, *ibid.*, **45**, 1398 (1923); Wobbe and Noyes (to be published in the near future).

Griffith and Shutt, *J. Chem. Soc.*, **119**, 1948 (1921).

Norrish and Rideal, *ibid.*, **125**, 2070 (1924).

Smith, *J. Amer. Chem. Soc.*, **47**, 1862 (1925).

²⁶ Daniels, *J. Amer. Chem. Soc.*, **48**, 607 (1926).

²⁷ Taylor, *ibid.*, **48**, 577 (1926).

Since we must eliminate the above simple equation as a means of calculating the active wave length, we may next inquire into the relation between absorption spectra and active wave lengths. We have not time in this review to enter into a detailed consideration of the various types of spectra. We may roughly divide spectra into band spectra and line spectra. Band spectra should be divided into many sub-groups. Nearly all substances possess absorption spectra in the infra-red. Some of these band systems have been accurately studied. There may be simple rotation bands. For the derivation of a formula to fit rotational bands it is assumed that $2 \pi M = m h$, where M is the impulse moment and is equal to the moment of inertia around the principal axis multiplied by the angular velocity, h is Planck's constant and m is a whole number. If I is the moment of inertia around the principal axis and ω is the angular velocity, then

$$I \omega = \frac{m h}{2 \pi}, E_{kin} = \frac{h^2}{8 \pi^2 I} m^2$$

If we put $B = \frac{h}{8 \pi^2 I}$ then if we imagine that a molecule absorbed light in such a manner that the value of m is changed to m' , the frequency will be given by

$$\nu = B (m'^2 - m^2)$$

This would be the simple formula for a pure rotation spectrum. Bands of this type would fall in the far infra-red.

In addition to simple increase in kinetic energy of rotation by absorption of infra-red radiation there may be an increase in the energy of vibration of the atoms with respect to each other. If the vibrations are those of a simple harmonic oscillator, the vibrations may be taken as of such a nature that the energy is some multiple of a simple quantum $h \nu_0$. If n' is the quantum number of the oscillation at the beginning and n is the quantum number at the end then the change in energy will be $h(n' - n) \nu_0$ and the change in wave number $(n' - n) \nu_0$. Combining with the

previous formula for rotation spectrum we find that the wave numbers should be represented by a formula of the type

$$\nu = (n' - n)\nu_0 + B(m'^2 - m^2)$$

There should be a series of bands of this type in the infra-red.

If we now consider the visible and ultra-violet bands it is necessary to consider another type of change in addition to rotation of the molecule and vibration of the atoms, namely electron displacements. The formula which fits this type of band is even more empirical than the formulae for bands in the infra-red. This formula is of the type

$$\nu = A(n, n') \pm 2B(n')m + C(n, n')m^2 + \dots$$

where

$$A(n, n') = \nu_0 + B(n') + n'\nu_0'(1 - n'x') - n\nu_0(1 - nx) + \dots$$

$$B(n') = \frac{1}{8\pi^2 I'} - \alpha n', \quad B(n) = \frac{1}{8\pi^2 I} - \alpha n.$$

$$C(n, n') = B(n') - B(n)$$

ν_0 is a term which depends on the electron displacement, m is a whole number, n and n' are whole numbers, I is the moment of inertia of the molecule after absorption and I' is the moment of inertia before absorption. Thus the formula contains three quantum numbers, n , n' and m and nine arbitrary constants ν_0 , ν_0' , x , x' , I , I' , α , α' . Unfortunately this is the type of band photochemists are most interested in studying. No satisfactory picture has yet been given of the rearrangements of the electron systems of molecules upon absorption. Consequently, while it is probably possible to calculate the change in energy of a molecule upon absorption of a given wave length, it is not possible to make any definite prediction as to the photochemical activity of a particular band. It would seem that more studies should be carried out using monochromatic light and substances whose band spectra have been thoroughly investigated.

Many substance in solution present what is called commonly "end absorption." This term means that absorption begins in the visible or near ultra-violet and continues down to the

shortest wave length studied, which is usually about the limit of the ordinary quartz spectrograph. This type of absorption is found for most substances in aqueous solution. Very rarely have instruments of sufficient dispersion been used in this type of work to permit developing the fine structure of these bands. Henri²⁸ has carried out many studies of the absorption spectra of organic substances and for certain ones, notably benzene and benzene derivatives, has been able to apply formulae of the type mentioned above.

The main conclusion we can draw from this brief discussion is that simple increase in rotation or in atomic vibration is not sufficient to produce the effects observed in photochemistry. It is necessary to use wave lengths whose effects on the molecule can best be interpreted by postulating changes in the electron system. The exact nature of the effects on the electron system are not known, although some idea of the difference in energy can be obtained.

In addition to band spectra line spectra are frequently observed as absorption spectra. In general it may be said that only monatomic gases (the rare gases and the metals) present well defined line absorption spectra. The polyatomic gases and practically all substances in solution present absorption spectra of the type discussed in the preceding paragraphs. We may take the Bohr atom as a basis for presenting a picture of the nature of line absorption spectra without going into detail as to the applicability or non-applicability of this atom-model to other fields. All atoms are supposed to have a certain arrangement of their planetary electrons when they are in the normal state. When energy is absorbed the arrangement of these electrons is changed. We are here concerned only with the outermost or valence electrons. If we designate the normal position of one of these electrons as "one," then when the atom absorbs energy the electron is changed to some other position further removed from the nucleus which we may call "two" or "three" or etc. If the electron were already in position "two" upon absorption it could

²⁸ Henri, *Etudes de Photochimie*, Gauthier-Villars et Cie, 1919; *La Structure des Molecules*, 1925.

go to position "three" or "four," etc. As a result the normal atom would only absorb in such a way that the electron would be removed from position "one" to some other state and the other lines observed in the emission spectrum and ascribed to the return of the electron from some state to state "two" would only be obtained in the absorption spectrum at high light intensities.

In general very little work has been done in photochemistry involving monatomic gases directly. Most of those monatomic gases which react at all react quite rapidly, and there would seem little possibility of determining accurately the effect of line absorption of this type. As a result of the researches of Cario and Franck,²⁹ however, it has been shown that mercury vapor may absorb light of wave length 2537, which corresponds to the first line in a series of the type mentioned above, and may transmit the energy upon collision to hydrogen or to other molecules. In the case of hydrogen the result is apparently dissociation and the monatomic hydrogen formed may react subsequently with molecules which will not react normally with diatomic hydrogen. There is some evidence that mercury in the first resonance state forms a hydride upon collision with a hydrogen molecule.³⁰ Whatever the mechanism of the process, hydrogen is made available for reactions for which ordinary hydrogen is not active. Cario and Franck studied the reaction of this monatomic hydrogen with certain metal oxides. Dickinson³¹ has studied the formation of water vapor from hydrogen and oxygen at 45°, Mitchell³² has studied this same reaction in some detail and has also studied the effect of the addition of an inert gas like argon. Taylor and Marshall³³ have carried out a series of reactions (with ethylene, oxygen, carbon monoxide and nitrous oxide). Noyes³⁴ has found that traces of ammonia are formed from nitrogen and hydrogen at the boiling point of mercury. It was not

²⁹ Cario and Franck, *Z. Physik*, **11**, 161 (1922); Franck, *ibid.*, **24**, 450 (1923).

³⁰ Compton and Turner, *Phil. Mag.*, **48**, 360 (1924).

³¹ Dickinson, *Proc. Nat. Acad. Sci.*, **10**, 409 (1924).

³² Mitchell, *ibid.*, **11**, 458 (1925).

³³ Taylor and Marshall, *J. Phys. Chem.*, **29**, 1140 (1925). See also Taylor, *Trans. Far. Soc.*, 1926 (Report of meeting held October 1, 1925).

³⁴ Noyes, *J., Amer. Chem. Soc.*, **47**, 1003 (1925).

definitely shown in this case, however, that there had been no effect either by multiple collision or by the silica walls on the nitrogen, since the pressure and temperature were higher than had been used by the other authors. Taylor³⁵ has shown that no ammonia is formed at lower temperatures. Recently Hirst³⁶ has studied a series of reactions in the presence of a mercury surface and radiation from a quartz mercury arc lamp. He finds that both ammonia and hydrazine are formed from hydrogen and nitrogen. Hirst is inclined to the view that the surface plays an important part in the reactions as carried out. In this connection it is of interest to point out some work by Franck and Grotrian³⁶ on the absorption band at 2540 Å of mercury vapor. Olson and Meyers³⁷ have studied the hydrogenation and polymerization of ethylene. They find that the initial rate of reaction is proportional to the square root of the hydrogen pressure. This they ascribe to the slow rate of diffusion of the monatomic hydrogen away from the proximity of the window of the reaction vessel, where most of the radiation would be absorbed. The work of Mitchell³² is in agreement with this assumption since he found that the presence of argon decreased the reaction rate.

As a result of these studies we may conclude that activation by collisions of this type may play a very important rôle in photochemistry and we may have here the explanation of the remarkable effect, in certain instances, of very small amounts of impurity.

We may summarize briefly the conclusions which may be drawn from this preliminary discussion. A great deal of the data in photochemistry is unreliable due to faulty technique and many of the conclusions should be regarded as empirical, no satisfactory explanation having been found. Certain reactions have been studied with sufficient detail to permit the postulation of mechanisms, but usually several mechanisms will prove equally satisfactory. In addition to the ordinary difficulties involved in the studies on kinetics of reactions, we have the difficulty of isolation

³⁵ Hirst, *Proc. Camb. Phil. Soc.*, **23**, 162 (1926).

³⁶ Franck and Grotrian, *Z. tech. Physik*, **3**, 194 (1922).

³⁷ Olson and Meyers, *J. Amer. Chem. Soc.*, **48**, 389 (1926).

of the effective wave length, the determination of the exact amount of radiation absorbed and the possibility that the entire effect may be due to small amounts of impurity. We have discussed the various general types of absorption spectra and have arrived at the general conclusion that some rearrangement of the electron systems of the molecules entering into reaction is necessary. The extremely interesting work which was started by Cario and Franck has thrown open a new method of attack on photochemical problems and we may look for new important results along this line in the future. In passing we may mention some calculations by Volmar³³ on the effective wave length necessary for the decomposition of certain organic compounds. By postulating dissociation as the activation process and assuming certain values for the energy necessary to break the various bonds, he is apparently able to calculate with some degree of success the wave lengths which will cause certain reactions to occur. The agreement is better than the accuracy of the data used in the calculations would warrant, although his calculations may prove of importance.

In what follows we have chosen certain photochemical reactions as typical and have presented a rather full discussion of the work on them. This list is by no means complete, nor is our selection necessarily the best that could be made. Allmand²² has covered the field in a very thorough and admirable manner and we would refer the reader to his summary should information of a more general nature be desired. We hope merely to show what conclusions have been reached with regard to typical photochemical reactions and to show something of the trend which photochemistry has taken. There are, of course, an enormous number of qualitative photochemical studies, particularly on organic reactions. These studies have consisted, usually, in exposing various systems to the sun's rays and determining after a given length of time what has happened. These studies may have some practical, but they have very little theoretical interest.

³³ Volmar, *Compt. rend.*, 178, 697 (1924).

PART II. TYPICAL PHOTOCHEMICAL REACTIONS

A. The decomposition of ozone

The decomposition of ozone, both thermally and photochemically, has been the subject of a number of investigations during the last twenty-five years. The thermal decomposition of partially ozonized oxygen has been studied by Warburg,³⁹ Clement,⁴⁰ Perman and Greaves,⁴¹ Jahn,⁴² and Chapman and Jones.⁴³ These workers all agree that in the absence of a catalyst the reaction is second order with respect to the ozone concentration. Jahn found that the presence of oxygen retarded the decomposition, while Chapman and Jones found that it had little effect. Clement measured the reaction rate at thirteen temperatures between 80° and 250°; from this data he arrives at the equation

$$\log k = - 5700/T + 14.939$$

The results of Warburg and of Perman and Greaves, which are less extensive, are in approximate agreement with this equation.

Perman and Greaves also investigated the effect of increasing the wall surface by adding glass tubing or glass wool; they found that the reaction took place largely on the surface, and that in the presence of a porous substance such as pipestems it became first order. Clarke and Chapman,⁴⁴ however, consider the reaction to proceed almost entirely in the gas phase. The results of Perman and Greaves may possibly be explained by imperfect drying of the glass wool, or by the presence of other impurities in it.

The photochemical decomposition was first investigated in ultra-violet light by Regener⁴⁵ and von Baur.⁴⁶ Both found the

³⁹ Warburg, *Ann. Physik*, **9**, 1286 (1902).

⁴⁰ Clement, *ibid.*, **14**, 341 (1904).

⁴¹ Perman and Greaves, *Proc. Roy. Soc.*, **80A**, 353 (1908).

⁴² Jahn, *Z. anorg. Chem.*, **48**, 260 (1906).

⁴³ Chapman and Jones, *J. Chem. Soc.*, **97**, 2463, (1910).

⁴⁴ Clarke and Chapman, *J. Chem. Soc.*, **93**, 1638 (1908).

⁴⁵ Regener, *Ann. Physik*, **20**, 1033 (1906).

⁴⁶ von Baur, *ibid.*, **33**, 598 (1910).

reaction to be of the first order in the ozone concentration. Weigert⁴⁷ found that for very thin layers the apparent order increased, reaching, in his experiments, 1.4 at a thickness of 5 mm. In the same paper Weigert postulates the photo-formation of "active centers" which cause chemical decomposition in some catalytic manner. On this basis he explains the observation of von Baur that the addition of an inactive gas retards the action; he suggests that the effect is to hinder diffusion of the active centers, thus decreasing their effectiveness.

Griffith and Shutt⁴⁸ have worked on the decomposition of ozone by visible light, using a set of Eastman color filters. They find two active regions, 5100–6150 Å and 6700–7600 Å. The methods they used are not capable of much accuracy and the limits of these regions are only approximate. Comparison of their results with the visible light absorption of ozone, as determined by Chappuis,⁴⁹ Schoene,⁵⁰ Liveing and Dewar⁵¹ and Ladenburg and Lehmann⁵² indicate that the photochemical efficiency of visible light is determined primarily by its absorption coefficient, since the two regions which are active photochemically are the only regions of visible absorption. Unfortunately, nothing is known about the quantum efficiency of visible light for this reaction.

The photochemical reaction has been studied in the presence of various other gases. Von Baur⁴⁶ found that upon replacing the oxygen in the mixture by an inactive gas the reaction was accelerated. Griffith and Shutt⁵³ and Weigert and Bohm,⁵⁴ the former using visible light, the latter ultra-violet, both find a specific acceleration due to hydrogen, much greater than for other gases. The study of this reaction is complicated by the simultaneous water formation and the results are little more than qualitative. There have been three explanations suggested for this hydrogen

⁴⁷ Weigert, *Z. physik. Chem.*, **80**, 78 (1912).

⁴⁸ Griffith and Shutt, *J. Chem. Soc.*, **119**, 1948 (1922).

⁴⁹ Chappuis, *Compt. rend.*, **91**, 935 (1880); **94**, 858 (1882).

⁵⁰ Schoene, *J. Russ. Chem. Soc.*, **2**, 250 (1884); *J. Chem. Soc.*, **48**, 713 (1885).

⁵¹ Liveing and Dewar, *Proc. Roy. Soc.*, **46**, 222 (1889).

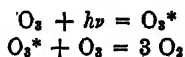
⁵² Ladenburg and Lehmann, *Verh. physik. Ges.*, **8**, 125 (1906).

⁵³ Griffith and Shutt, *J. Chem. Soc.*, **123**, 2752 (1923).

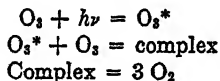
⁵⁴ Weigert and Bohm, *Z. Physik. Chem.*, **90**, 194, 223 (1915).

catalysis. Weigert and Bohm proposed local heating due to the reaction $O_3 + H_2 = H_2O + O_2$. Griffith and Shutt suggest catalysis due to the water formed; Warburg⁵⁵ found this effect to exist, when ultra-violet light is used, but his work shows it to be insufficient in magnitude to account for the whole acceleration observed. Hence Griffith and Shutt⁵³ reject this explanation and propose activation of the ozone by collision with activated water molecules, formed by $H_2 + O = H_2O^*$ or $H_2 + O_3 = H_2O^* + O_2$. Both of these reactions are considerably exothermic.

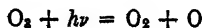
Recent work on the effect of various inert or inactive gases on the decomposition is considered by Griffith and McKeown.⁵⁶ On the basis of the catalytic effect of such gases they reject the mechanism previously given by Griffith and MacWillie⁵⁷ for the visible light reaction:



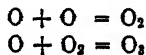
They propose instead



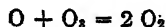
By postulating such an intermediate product, they are able to explain the catalytic effect of inert gases as due to their action in decomposing the complex into O_2 . Griffith and McKeown criticize the mechanism given by Warburg⁵⁵ for the ultra-violet reaction for its failure to explain this catalysis. This mechanism is



followed by



or



⁵⁵ Warburg, *Sitzungsber. Preuss. Akad.*, **1913**, 644.

⁵⁶ Griffith and McKeown, *J. Chem. Soc.*, **127**, 2086 (1925).

⁵⁷ Griffith and MacWillie, *ibid.*, **123**, 2767 (1923).

When the mixture $O_3 - O_2 - Cl_2$ is studied, more satisfactory results are obtained. Here the decomposition takes place in blue light, and the chlorine is left unchanged. Weigert⁵⁸ showed the reaction velocity to be proportional only to the amount of light absorbed and Bonhoeffer,⁵⁹ using the mercury line 4360 and the group at 4060, confirmed the results of Weigert and showed that, for pressures of O_3 greater than 0.5 mm. of mercury, two molecules of ozone are decomposed per quantum absorbed. The Einstein law is thus verified for this reaction.

Bonhoeffer found, at the same time, that if bromine replaces chlorine as a sensitizer, the quantum efficiency rises from two to thirty-one.

Weigert⁶⁰ has discussed the photochemical chlorine reactions, and especially the ozone decomposition, in considerable detail. He points out the large effect of small amounts of matter on the final distribution of the light energy absorbed. In the presence of traces of water, this energy becomes transformed into heat (the Budde effect) while in the presence of traces of O_3 it is changed into chemical energy. Weigert compares the photo-sensitization of O_3 by chlorine with the dissociation of hydrogen by mercury vapor which is absorbing its resonance radiation. He calculates that, for these two reactions, when the pressures of H_2 on the one hand and of O_3 on the other are just large enough to have no further effect on the reaction, that layers of the two reacting mixtures which absorb the same amount of light contain about equal numbers of molecules of the two reactants. In the Cario-Franck activation of H_2 , it is exceedingly probable that the first collision of an activated Hg atom will be with a hydrogen molecule; for the ozone chlorine mixture, however, an activated molecule of chlorine must, on the average, undergo 1000 collisions before striking an ozone molecule. This has lead Bonhoeffer,⁵⁹ Noddack,⁶¹ Grüss⁶² and others to assume that an activated molecule may pass unchanged through collisions with an inactive one.

⁵⁸ Weigert, *Z. Elektrochem.*, **14**, 591 (1908).

⁵⁹ Bonhoeffer, *Z. Physik*, **13**, 94 (1923).

⁶⁰ Weigert, *Z. physikal. Chem.*, **106**, 407 (1923).

⁶¹ Noddack, *Z. Elektrochem.*, **27**, 359 (1921); *Physik. Zeit.*, **21**, 602 (1922).

⁶² Grüss, *Z. Elektrochem.*, **29**, 144 (1923).

Weigert points out that, by destroying the mechanism that has been given for transformation of radiation into random kinetic energy, i.e., heat, this hypothesis would greatly diminish the value of the entire concept of energy transfer during collisions. He postulates instead a weak resonance radiation for chlorine and finds confirmation for this idea in the observation of Halban and Siedentopf⁶³ that while wet and dry chlorine have the same absorption, only the wet shows the Budde effect. This idea has been criticized by Allmand,⁶⁴ who realizes the difficulties involved in the long life for activated molecules, but does not consider Weigert's idea more plausible.

Finally mention must be made of an attempt by W. C. McC. Lewis⁶⁵ to apply the simple radiation hypothesis to the ozone decomposition. The work of Clement⁴⁰ and others on the thermal decomposition, mentioned previously, indicates a heat of activation of 26,000 calories. Lewis takes half of this as the heat of activation of a single molecule; this does not seem to be justified unless every molecule of ozone that takes part in the reaction must be activated, which is not in accord with most of the mechanisms which have been suggested. The wave length corresponding to 13,000 calories is 21,700 Å, that to 26,000 calories is 10,850 Å. O₃ has an absorption band at about 47,500 Å^{66,67}. Then, according to Lewis, two quanta of this long wave length are absorbed to produce activation. Lewis considers also the higher stages of activation corresponding to frequencies that are known to be effective. Thus, it is probable that O₂ is dissociated by light of wave length 1850, to which corresponds E_{O₂} = 151,000 calories. The heat of formation of ozone is not accurately known, but is about 35,000 calories^{68,69}. Then, if E_{O₃} is the energy necessary for the reaction O₃ = O₂ + O, the equation

$$- 35000 = E_{O_3} - 1/2 E_{O_2}$$

⁶³ Halban and Siedentopf, *Z. Elektrochem.*, **28**, 499 (1923).

⁶⁴ Allmand, *Trans. Far. Soc.*, report of meeting held Oct. 1, 1925.

⁶⁵ W. C. McC. Lewis, *Trans. Far. Soc.*, **17**, 373 (1922).

⁶⁶ Warburg and Leithauser, *Ann. Physik*, **28**, 313 (1909).

⁶⁷ Angstrom, *Arkiv för Matematik (Stockholm)*, **1**, 347, 395 (1904).

⁶⁸ Meulen, *Rec. trav. chim.*, **2**, 69 (1883).

⁶⁹ Kailan and Jahn, *Z. anorg. Chem.*, **68**, 243 (1910).

may be written. Whence $E_{O_3} = 40,500$ calories. This corresponds to 7000 \AA and is doubtless in better agreement with the results of Griffith and Shutt on visible light decomposition than the accuracy of the data warrant. Lewis also explains the ultra-violet decomposition by a similar sort of calculation. Many other cases are known where discrepancies between active frequencies and those calculated from temperature coefficients may be explained by such calculations as these. In practically every case, however, the frequency calculated directly from the temperature coefficient corresponds to a region in the infra-red that is not active photochemically, frequently one that is not even absorbed.

In conclusion of the discussion of ozone decomposition, it must be mentioned that any complete theory of this reaction must explain the markedly different effects of chlorine and bromine on the decomposition. So far as we know, this has not been even attempted.

B. The decomposition of nitrogen pentoxide

The decomposition of nitrogen pentoxide is the only reaction known which certainly proceeds as a homogeneous, uncatalyzed first order gas reaction. The thermal reaction has been studied by Daniels and his co-workers,⁵ by Tolman and White⁷⁰ and by Hirst.⁵ It has been established by all of these workers that the reaction proceeds with a velocity dependent only upon the temperature. The velocity is the same in almost pure N_2O_5 as it is in the presence of large quantities of the decomposition products, N_2O_5 , NO_2 and O_2 . Indeed, dilution with 1000 parts of oxygen or nitrogen does not alter the specific reaction rate. It was thought at one time that the reaction was auto-catalytic, being affected by NO_2 , but this idea has been completely disproved.

This reaction, then, is particularly suitable for testing those theories which seek to connect the energy of activation, as calculated from the temperature coefficient of the reaction rate, with the photochemically active frequencies. The wave length cor-

⁷⁰ Tolman and White, J. Amer. Chem. Soc., 47, 1240 (1925).

responding to the active frequency ν , calculated from the simple equation

$$\frac{d \ln k}{dT} = \frac{N h \nu}{RT^2}$$

is 1.16μ . The work of Warburg and Leithauser⁷¹ shows that nitrogen pentoxide does not absorb light of this wave length, but does have bands at 3.39 and 5.81μ . This led Lewis⁷² to postulate that the activation occurs through absorption of five quanta corresponding to 5.81μ or more probably of three corresponding to 3.39μ . Either of these processes would give the proper energy. The work of Daniels and Johnston⁵ has shown the originally calculated frequency, corresponding to 1.16μ , to be inactive photochemically while more recently both Daniels²⁸ and Taylor²⁷ have shown that pure nitrogen pentoxide is unaffected by any infra-red radiation. Indeed, the longest wave length at which any photochemical activity has been observed is 4600 \AA ⁵ and for that the presence of NO_2 is necessary.

In view of the remarkable constancy of the specific reaction rate for this decomposition, not only in the gaseous phase but in solution,⁷³ it seems hardly possible that molecular collisions have anything to do with the reaction. The simple radiation theory is certainly not capable of explaining it. Indeed, if there is any frequency of radiation that is responsible for the thermal reaction, it is hard to see in what part of the spectrum it can lie. The infra-red waves longer than 8μ would hardly have enough energy to be effective. Between 8μ and $460 \text{ m}\mu$ the light has been shown to be without effect. Blue light is active in the presence of NO_2 , but it has been shown that this substance does not affect the thermal reaction and hence this is not the active region; certainly ultra-violet light is not present in sufficient density in the thermal radiation at ordinary temperatures to account for the observed rate of reaction, unless reaction chains are set up; but this last supposition is very unlikely in view of the constancy of the specific reaction rate for widely varying pressures.

⁷¹ Warburg and Leithauser, *Ann. Physik*, **23**, 313 (1909).

⁷² Lewis, *Trans. Far. Soc.*, **17**, 535 (1922).

⁷³ Lueck, *J. Amer. Chem. Soc.*, **44**, 757 (1922).

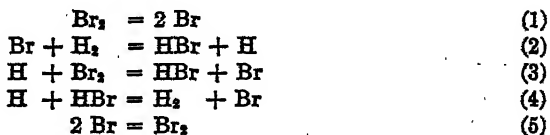
Similar results are obtained from a study of other reactions.²⁵ It is now certain that the original, so-called simple radiation hypothesis must be abandoned. Furthermore, in spite of the extensions which have been suggested by Lewis and Smith, Tolman² and others, it is still far from clear how thermal reactions are to be explained as basically photochemical. It is only the apparently greater difficulties^{2, 74} that an explanation on the basis of molecular collisions must overcome, which encourage further efforts to improve upon the radiation hypothesis.

C. The decomposition and formation of hydrogen bromide

The thermal formation of hydrogen bromide from its elements was studied by Bodenstein and Lind⁷⁵ who found that between 200° and 300° the rate of reaction was given by the equation.

$$\frac{d [2 \text{ HBr}]}{dt} = \frac{k [\text{H}_2] \sqrt{[\text{Br}_2]}}{m + \frac{[2 \text{ HBr}]}{[\text{Br}_2]}}$$

The fact that the bromine concentration appears as a square root was supposed by them to indicate that bromine atoms were necessary for the reaction. The inhibiting effect of the hydrogen bromide formed was not understood at the time. Somewhat later, Christiansen,⁷⁶ Polanyi,⁷⁷ and Herzfeld⁷⁸ proposed explanations for this action. The essential part of the three explanations is the same:



The reactions (1) and (5) together maintain a concentration of bromine atoms which is always proportional to the square root of the bromine concentration, since the other reactions, (2), (3),

⁷⁴ Christiansen and Kramers, *Z. physik. Chem.*, **104**, 451 (1923).

⁷⁵ Bodenstein and Lind, *Z. physik. Chem.*, **57**, 168 (1906).

⁷⁶ Christiansen, *Dansk. Vid. Math. Phys. Med.*, **1**, 14 (1919).

⁷⁷ Polanyi, *Z. Elektrochem.*, **26**, 50 (1920).

⁷⁸ Herzfeld, *ibid.*, **25**, 301 (1919); *Ann. Physik*, **59**, 635 (1919).

and (4) do not reduce the number of bromine atoms; it seems that these authors neglect the effect of the reaction $2\text{H} = \text{H}_2$. However, if either $[\text{Br}_2]$ or $[\text{HBr}]$ is large compared to $[\text{H}]$, this neglect would not introduce an appreciable error.

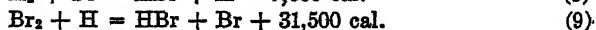
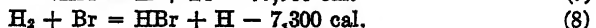
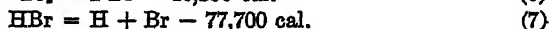
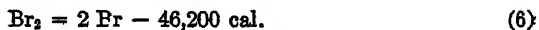
On the basis of the first five equations, an expression for the rate of reaction is found which is of the same form as that experimentally determined.

The photochemical formation of hydrobromic acid has been studied by Bodenstein and Lütke Meyer.⁷⁹ Here the velocity follows the equation

$$\frac{d[2\text{HBr}]}{dt} = \frac{k[\text{H}_2]\sqrt{I}}{m + \frac{[2\text{HBr}]}{[\text{Br}_2]}}$$

where I is the amount of light energy absorbed. The constant k is not necessarily independent of the spectral region considered. The rate of formation of bromine atoms by light is doubtless proportional to the amount of light absorbed, for constant wavelength; if this is the case, the concentration of bromine atoms in the steady state will be proportional to the square root of the amount of light absorbed. This makes it seem probable that the initial photochemical reaction is the dissociation of bromine and that the succeeding steps are the same as in the thermal reaction.

This dissociation mechanism for photochemical reactions has been considered by Nernst,⁸⁰ Noddack, and by Warburg.⁸¹ Bowen⁸² has attempted to develop a complete theory of the photochemistry of the hydrogen halides on the basis of such a picture. Thus from the heats of dissociation of hydrogen (which he takes as 85,000 calories) and of Br_2 (46,200 calories) and the heat of formation of hydrogen bromide he writes



⁷⁹ Bodenstein and Lütke Meyer, *Z. physik. Chem.*, **114**, 208 (1924).

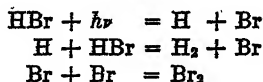
⁸⁰ Nernst, *Z. Elektrochem.*, **24**, 355 (1918). Nernst and Noddack, *Sitz. Preuss. Wiss.*, **110** (1923).

⁸¹ Warburg, *ibid.*, **1916**, 300, 314.

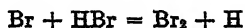
⁸² Bowen, *J. Chem. Soc.*, **125**, 1233 (1924).

The energy necessary to dissociate bromine corresponds to 6100 Å; since this wave length is absorbed by bromine, Bowen considers that it must be the photochemical threshold for the formation of hydrogen bromide. Bowen then supposes that reaction (8) in spite of its slightly endothermic character, can occasionally occur; when it does, (9) may also take place. Since (8) is endothermic, it would be probably favored by increase in temperature; this is apparently the case since the photochemical reaction has the high temperature coefficient of 1.5.⁸³ The quantum efficiency is certainly very low at room temperature.⁸³ The reaction has been studied up to about 250°. Lind⁸⁴ working at that temperature, reports an efficiency still less than one. His work, however, does not seem to have been very extensive.

On the basis of equation (7), Bowen predicts a photochemical threshold at 3630 Å for the photolysis of hydrogen bromide. Hydrogen bromide does not absorb at this wave length. This reaction has been studied by Warburg⁸⁵ at the wave lengths 2090 and 2530. The quantum efficiency in both cases was 2.0 within the experimental error. Warburg has proposed for this reaction the mechanism



The reaction



can not take place, since it occurs with the absorption of about 30,000 calories. Bowen has proposed the same mechanism.

Bowen has applied this same type of mechanism to the photochemistry of HCl and HI. The results are in reasonably good agreement with experiment. There are two objections to which the theory is open. One is that the experimental values for the heats of dissociation, particularly of hydrogen and of chlorine, are

⁸³ Pusch, *Z. Elektrochem.*, **24**, 336 (1918).

⁸⁴ Lind, *J. Phys. Chem.*, **28**, 55 (1924).

⁸⁵ Warburg, *Sitzb. Preuss. Akad. Wiss.*, 1916, 314.

not known with any accuracy; the other is the possibility that the application of thermodynamics in this way to atomic processes is not justified. This latter suggestion has been made by many writers.

Coehn and Stuckardt⁸⁸ have studied both the formation and the decomposition of hydrogen bromide in quartz, in uviol and in glass vessels. They find that when light is used with a short wave limit of 2200, there is complete decomposition of the HBr; for a short wave limit of 3000 there is no decomposition and the formation is eventually complete, though the reaction is very slow. For intermediate wave lengths, about 2540, there is an equilibrium set up; in their particular experiments this equilibrium corresponded to about twenty per cent decomposition. This is in good agreement with the fact that HBr begins to absorb about 2640 Å.

There would be no particular reason for believing that the mechanism of the HBr formation is based upon a dissociation of bromine into atoms, were it not for the work of Bodenstein and Lind. Their results indicate fairly definitely that the thermal reaction takes place as a result of dissociation and they seem to show that the photochemical reaction takes place in the same way. There would seem to be no definite proof that this is the mechanism. For the decomposition, there is no reason for favoring the dissociation mechanism. Warburg has shown that it does not disagree with the facts, but it would be easy to explain the same results by means of a primary photochemical process consisting of activation.

D. The photochemical reactions of solids

With the exception of work on the silver halides with special reference to photographic processes, very little work has been done on the photochemistry of solid bodies. The problem of measuring the amount of light absorbed is, of course, a very difficult one to solve.

⁸⁸ Coehn and Stuckardt, Z. physik. Chem., 91, 722 (1916).

Renz⁸⁷ has worked on the darkening of thallous chloride and the reactions of cerium dioxide. These studies seem to have led to very little of a definite nature.

Bružs⁸⁸ has studied the thermal decomposition of a series of carbonates (ferrous, lead, cobalt, zinc, and mercurous). The variations in the critical increments for these decompositions are quite large, although the average is about 80,000 calories and Bružs concludes that this value is characteristic of the carbonate ion. This would correspond to a wave length of about 3600 Å. Schaefer and Schubert⁸⁹ have found that carbonates exhibit characteristic absorption at 6.5, 11.5 and 14.5 μ .⁹⁰

Berthelot and Gaudechon⁹¹ have studied both the thermal and photochemical decomposition of oxalic acid. They postulate that oxalic acid gives carbon dioxide and formic acid and that long waves change the latter into carbon monoxide and water and short waves into carbon dioxide and hydrogen. Noyes and Kouperman and Wobbe and Noyes²⁵ find that the wave length calculated from the critical increment does not agree with the wave length which makes the anhydrous solid decompose. The presence of water apparently accelerates the reaction considerably.

⁸⁷ Renz, *Helvetica Chim. Acta*, **2**, 704 (1919); *Zeit. anorg. allgem. Chem.*, **110**, 153 (1920).

⁸⁸ Bružs, *J. Phys. Chem.*, **30**, 680 (1926).

⁸⁹ Schaefer and Schubert, *Ann. Physik*, **50**, 283 (1916).

⁹⁰ There would seem to be a mistake in the footnote of the article by Bružs (page 693). The critical increment as calculated from the three wave lengths together would be 8,800 calories (not 89,000). This is one-tenth of the value found from the thermal decomposition.

⁹¹ Berthelot and Gaudechon, *Compt. rend.*, **158**, 1791 (1914).

HOMOGENEOUS REACTIONS

C. N. HINSHELWOOD (Oxford)

THE FUNDAMENTAL QUESTION

We observe chemical changes of all kinds to go on and to lead to states of equilibrium. The study of these states of equilibrium is, roughly speaking, the affair of thermodynamics. But the rate at which equilibrium is attained varies to an unlimited extent. The object in studying what we call chemical kinetics is to arrive at an understanding of why molecules undergo chemical change, and to be able to predict the rate at which a given chemical reaction will take place under definite conditions. To realize that rate of chemical change is a significant matter it is only necessary on the one hand to consider the vast industrial processes depending on the catalysis of gas reactions, or on the other hand to reflect on the instability of all living matter, which makes large provinces of biochemistry an affair of reaction velocity. But before it is possible to predict rates of reaction for the benefit of these applied sciences, or indeed before we can say whether the problems they put are soluble at all, we have first to investigate the purely theoretical question of what actually happens in the act of chemical transformation. For this purpose, the study of simple reactions in the gaseous state offers most hope of success, because all the resources of the kinetic theory of gases can be drawn upon in aid of the enquiry.

Now in a survey of all the simple gaseous reactions which have been examined experimentally, we find that they fall into two fundamentally distinct classes, namely homogeneous reactions and heterogeneous reactions. In homogeneous reactions the chemical change takes place among molecules flying about freely in the gas phase; in heterogeneous reactions the chemical change takes place in contact with the wall of the vessel in which the gas is contained, or at the surface of some solid substance added

to the system. The criterion of a homogeneous reaction is that its rate is independent of the extent of the solid surface exposed to the gas, while in a heterogeneous reaction the rate is directly proportional to the surface area.

Homogeneous gaseous reactions fall further into classes according to their "*order*," that is to say, according to the number of molecules participating in each individual act of chemical transformation. The order of a reaction is easily found by examining the effect of change of pressure on the velocity. Since, for example, the velocity of decomposition of ozone is proportional to the square of the partial pressure of the ozone, it follows from elementary considerations of probability that two molecules must come together before chemical change can occur. The process involved is thus $2\text{O}_3 = 3\text{O}_2$ and not $\text{O}_3 = \text{O}_2 + \text{O}$ which might have been the primary reaction, and have been followed by a rapid combination of the odd oxygen atoms. Unimolecular, bimolecular and termolecular gaseous reactions are known, but none of higher order.

A similar classification could be made of heterogeneous reactions. When, for example nitrous oxide decomposes at the surface of platinum or gold the primary process is $\text{N}_2\text{O} = \text{N}_2 + \text{O}$ and not $2\text{N}_2\text{O} = 2\text{N}_2 + \text{O}_2$, which is the reaction taking place in the gas phase. But the real order of a heterogeneous reaction cannot be inferred from the effect of pressure on the velocity unless certain special conditions are fulfilled which require fuller consideration.¹

ACTIVATION

In general, the act of chemical transformation of molecules depends upon a process known as "*activation*." This involves the acquirement by the molecule or molecules of an amount of energy considerably above the average. The exact nature of this molecular energy—whether kinetic energy or internal energy of some kind—it is not necessary in the first instance to specify. Nor is the means by which the energy is acquired relevant immediately.

¹ For a discussion of this see *The Kinetics of Chemical Change in Gaseous Systems*, Hinshelwood, Clarendon Press, Oxford, 1926, p. 155.

Under different circumstances and in different types of reaction the energy of activation may be of different kinds; it may sometimes be communicated to the molecule by a collision with another molecule or be received as a result of the absorption of some kind of radiant energy. Activation is an absolutely general process and is necessary both in homogeneous and in heterogeneous reactions. It forms the basis of the whole kinetic theory of reaction velocity. We may say that it is a necessary condition for chemical change that the molecule or molecules participating in the transformation should possess energy in excess of a certain minimum known as the *energy of activation*. The evidence upon which this statement is based is as follows.

1. In a chemical reaction which proceeds with measurable velocity only exceptional molecules are at any moment in a position to undergo transformation. This applies to reactions of the most diverse kinds. In a homogeneous unimolecular reaction, such as the decomposition of nitrogen pentoxide, it is obvious, since otherwise the molecules would all react at once. In a bimolecular change, such as the combination of hydrogen and iodine, it is easy to calculate the number of collisions taking place at a given temperature and pressure between molecules of hydrogen and molecules of iodine, and to show that only one of these in many millions leads to interaction. Again when oxygen at low pressure acts upon a hot tungsten wire, a very small fraction only of the molecules which strike the wire are able to react with it.²

2. Further the attainment of this exceptional reactive state is very markedly favoured by increase of temperature. The velocity constant of a reaction varies with temperature according to the Arrhenius equation³ $d \log k / dT = E/RT^2$. This represents a very much more rapid increase than could be accounted for by such factors as increased collision frequency. Arrhenius himself suggested the existence of "inactive" and "active" molecules in equilibrium, the active molecules being formed endothermically from the inactive molecules. The increase of reaction velocity with temperature he attributed to the normal shift in

² Langmuir, J. Amer. Chem. Soc., 35, 105, 931 (1913); 41, 167 (1919).

³ Arrhenius, Z. physik. Chem., 4, 226 (1889).

the position of the equilibrium between active and passive molecules in accordance with the second law of thermodynamics. The quantity E of the equation would thus represent the heat of formation of the active molecules. The essential points of this theory are now generally accepted but expressed in rather a different way. We do not think of the active molecule as a sort of tautomeric form of the normal molecule, but merely as a normal molecule of exceptionally high energy content. In terms of the Bohr theory it can sometimes be regarded as a molecule in one of its higher quantum states, although it is not necessary in the first instance to formulate any precise views about this. The objection to thinking about the active molecule as a definite tautomeric form is that it would be very difficult to imagine what the tautomeric form could be in such simple reactions as the decomposition of ozone or of hydrogen iodide. That tautomeric change sometimes intervenes as one of the stages in more complex reactions, such as those of organic chemistry, is quite irrelevant. There is no reason why it should not. But it is not a fundamental characteristic of chemical change. An active molecule of hydrogen iodide has a structure no different from that of an ordinary one.⁴

The characteristic form of the relation between reaction velocity and temperature now follows at once from the equations of the kinetic theory, which express the distribution of energy among the molecules of a gas. At a given temperature the molecules have a certain average kinetic energy, a certain average rotational energy and so on. Some have much more than the average amount of a given kind, some much less. The exact form of the "distribution law" varies according to the kind of energy under consideration, but the following law is approximately true of all forms. If N be the total number of molecules, then the number N' for which the energy of one particular kind, for example rotational, exceeds an amount ϵ is given by $N' = N e^{-\epsilon/kT}$ where k is the gas constant, R , divided by Avogadro's number. If instead

⁴ Compare Rice, Fryling and Weselowski, *J. Amer. Chem. Soc.*, **46**, 2405 (1924). According to the present writer such views can only be applicable to quite special cases. See 1, p. 90.

of ϵ , the energy per molecule we write E , the energy per *gram* molecule, this becomes $N' = Ne^{-E/RT}$. The factor $e^{-E/RT}$ thus represents the probability that a molecule possesses energy of some given kind in amount greater than E , E being reckoned per gram molecule. Since the velocity of reaction is proportional to the number of activated molecules we have for the velocity constant

$$k = \chi e^{-E/RT}$$

The factor χ differs in form according to the nature of the reaction. In a bimolecular reaction, for example, it must involve the number of collisions between molecules of the right kind; in unimolecular reactions, on the other hand, it may be independent of the collision number, and may possibly involve the rate at which radiant energy can be transferred. To a first approximation it is independent of temperature—or at any rate its variation is negligible in comparison with the very rapid rate of change of the exponential factor $e^{-E/RT}$. Treating it therefore as constant and differentiating logarithmically with respect to T , we obtain the Arrhenius equation. Precisely analogous considerations, although slightly more complicated, apply to heterogeneous reactions.⁵

WHAT CONSTITUTES A SOLUTION OF THE FUNDAMENTAL PROBLEM OF CHEMICAL KINETICS?

In the light of what has just been said let us return for a moment to the question of the possibility of calculating reaction rates absolutely. We have seen that reactive molecules are represented by a fraction approximately equal to $e^{-E/RT}$ of the total number. E can be found from the temperature coefficient of the reaction velocity by application of the Arrhenius equation. The question arises whether we can hope to calculate it *a priori*, and the answer must be that the attempt is definitely outside the domain of chemical kinetics. Since it represents the energy which must be communicated to a molecule in order to render the parts capable of re-arrangement, its absolute calculation is obviously

⁵ Loc. cit., 1, p. 172 et seq.

a problem of molecular structure. A thermodynamic analogy may help at this point. The laws of thermodynamics enable us to predict with accuracy the shift of chemical equilibria with temperature, and even the absolute position of these equilibria, provided that we know the heat of reaction and certain other thermal properties of the system under consideration. But they do not under any circumstances relieve us from the necessity of finding out by quite independent means what the heat of reaction is in a given instance. To calculate heats of reaction would be a very difficult problem in molecular mechanics, which at the present time we should hardly think of attempting. In the study of kinetics the heat of activation, E , plays a part analogous to heat of reaction in the study of equilibria. It is a specific molecular property which must be determined for each reaction we study before further progress can be made. The legitimate scope of enquiry is then: given the heat of activation what is the absolute reaction velocity? This question we may hope to answer, and indeed a good deal of progress has already been made. In the equation.

$$\text{Rate of reaction} = \chi e^{-E/RT}$$

we must realize that E is the fundamental datum, determinable from the temperature coefficient, but the theoretical calculation of which belongs to the larger province of the relation between structure and physical constants. Our problem is to investigate the nature of χ in reactions of various types.

We will now review the various kinds of homogeneous reaction in order.

BIMOLECULAR REACTIONS

It is most convenient to start with the consideration of bimolecular reactions, because the theory of these has been more fully worked out than that of unimolecular and termolecular reactions. The following homogeneous bimolecular reactions are known: The decomposition of hydrogen iodide,* $2\text{HI} = \text{H}_2 + \text{I}_2$; the

* Bodenstein, *Z. physik. Chem.*, **29**, 295 (1899). In connection with some criticisms of H. A. Taylor, *J. Amer. Chem. Soc.*, **28**, 984 (1924), see ref. 1, p. 49.

union of hydrogen and iodine,⁷ $\text{H}_2 + \text{I}_2 = 2 \text{HI}$; the decomposition of nitrous oxide,⁸ $2\text{N}_2\text{O} = 2\text{N}_2 + \text{O}_2$; of ozone,⁹ $2\text{O}_3 = 3\text{O}_2$; and of acetaldehyde in the gaseous state,¹⁰ $2\text{CH}_3\text{CHO} = 2\text{CO} + 2\text{CH}_4$; the decomposition of chlorine monoxide,¹¹ $2\text{Cl}_2\text{O} = 2\text{Cl}_2 + \text{O}_2$ (ultimately), although attended with certain complications is also an essentially bimolecular change. All these have been fairly thoroughly investigated. The union of nitrogen and oxygen to nitric oxide,¹² and the decomposition of nitric oxide¹³ have been stated to be bimolecular, but the experimental results, obtained by the rather unsatisfactory streaming method, are not quite unambiguous and are unsuitable for calculations, there being some doubt as to the homogeneous or heterogeneous nature of the reactions under the conditions of the investigations.

Bimolecular reactions depend upon collisions between two molecules. The number of collisions taking place in 1 cc. in unit time between like molecules can be calculated from the kinetic theory by the equation

$$\text{Number of collisions} = Z = \sqrt{2} \pi \sigma^2 n^2 \bar{u}$$

where σ = molecular diameter

\bar{u} = root mean square velocity of the molecules

n = number of molecules in 1 cc. at the given temperature and pressure.

σ is calculated from viscosity data, and since viscosity is a phenomenon directly concerned with the mean free path and therefore with the collision number, any arbitrariness in our definition of the diameter of a molecule does not in the least affect the validity of the formula. Thus we can calculate the number of collisions with great certainty as to the order of magni-

⁷ Bodenstein, loc. cit., 6.

⁸ Hunter, *Z. physik. Chem.*, **53**, 441 (1905). Hinshelwood and Burk, *Proc. Roy. Soc., A*, **106**, 284 (1924).

⁹ Chapman and Jones, *J. Chem. Soc.*, **97**, 2463 (1910).

¹⁰ Hinshelwood and Hutchison, *Proc. Roy. Soc., A*, **111**, 380 (1926).

¹¹ Hinshelwood and Prichard, *J. Chem. Soc.*, **123**, 2730 (1923). Hinshelwood and Hughes, *J. Chem. Soc.*, **125**, 1841 (1924). The results were confirmed in Bodenstein's laboratory. *Z. physik. Chem.*, **118**, 372 (1925).

¹² Nernst, *Z. anorg. Chem.*, **49**, 213 (1906).

¹³ Jellinek, *Z. anorg. Chem.*, **49**, 229 (1906).

tude, although not with minute accuracy. It is to be noted that all molecular diameters are of the order 10^{-8} cm.

As we have said, the number of collisions turns out to be many millions of times greater than the number of molecules which react. This was one of the reasons for supposing activation to be necessary. Suppose one of the colliding molecules requires energy greater than E_1^* and the other energy greater than E_2 . The fractions of the total number having energies respectively greater than these amounts are $e^{-E_1/RT}$ and $e^{-E_2/RT}$ approximately. Thus of all the collisions, the fraction in which the two molecules satisfy the postulated condition is $e^{-E_1/RT} \times e^{-E_2/RT}$ or $e^{-(E_1+E_2)/RT}$. If we write $E = E_1 + E_2$ this means that a fraction $e^{-E/RT}$ of the collisions is between active molecules. The rate of reaction should be proportional to the total number of collisions multiplied by this factor. Thus number of molecules reacting = $A \cdot Z \cdot e^{-E/RT}$.

We know nothing about A except that it is independent of temperature. The internal economy of molecules, for all we know to the contrary, might be such that only one collision in many millions could be effective even when the condition of sufficient energy is satisfied. If, to take a rough and ready analogy, we liken the energy of activation to the effort necessary to drag open the heavy door of a safe, then the factor A represents the chance of finding the right combination for first opening the lock, a process which involves no expenditure of energy, but without which no expenditure of energy produces any effect. We shall see, however, that in bimolecular reactions the combination of the molecular locks is of an extremely primitive kind, and that the energy condition is the only important one.

Without knowing anything about A we can find E for, since Z is proportional to $T^{\frac{1}{2}}$, we have

$$k = \text{constant} \cdot \sqrt{T} \cdot e^{-E/RT}$$

whence, taking logarithms and differentiating, we have

$$d \log k / dt = E/RT^2 + 1/2T$$

* E_1 and E_2 are calculated per *gram* molecule.

E can thus be found from the ratio of the velocities at two temperatures, and we can then substitute in the former equation and find A. Experiments show that in all known instances of bimolecular reactions the expression—number of collisions $\times e^{-E/RT}$ —comes out *nearly* equal to the actual number of molecules observed to react.¹⁴ Thus no other *important* factor beyond the possession of the energy is necessary as a condition for reaction.

The consequence of this striking result is very clearly shown by comparing the different bimolecular reactions among themselves. Since all molecular diameters are of about the same magnitude, the collision number does not greatly vary between one gas and another. Moreover, if no important condition except the energy condition has to be satisfied, then the rate of a bimolecular reaction can be seen from the equation to be determined mainly by the value of the exponential factor $e^{-E/RT}$. Different bimolecular reactions have very different values of E. The greater the value of E the smaller the factor $e^{-E/RT}$, and therefore the smaller the reaction rate, or, looked at in another way the higher the temperature at which the rate of reaction will attain any given value. This expectation is confirmed in a very striking way.¹⁵ For example, the energy of activation in the chlorine monoxide decomposition is 21,000 calories; that in the decomposition of hydrogen iodide is 44,000 calories. The latter reaction should therefore go much more slowly, as indeed it does. The two rates should be approximately equal at temperatures such that E/RT is the same for each. It is in fact found that the decomposition of hydrogen iodide proceeds at the same rate at 760° abs. as that of chlorine monoxide at 384° abs. Thus the absolute temperatures are almost exactly in the ratio of the heats of activation. Again, hydrogen iodide and acetaldehyde decompose at approximately the same rate at the same temperature. In accordance with this we find that their heats of activation are nearly identical, that of acetaldehyde being 45,500 calories. If

¹⁴ W. C. McC. Lewis, J. Chem. Soc., 113, 471 (1918). Dushman, J. Amer. Chem. Soc., 43, 397 (1921).

¹⁵ Hinshelwood and Hughes, J. Chem. Soc., 125, 1841 (1924). Hinshelwood and Thornton, Phil. Mag., 50, 1135 (1925).

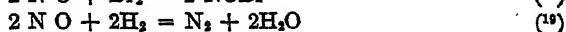
specific factors of the kind indicated by the analogy of the combination lock played any important part, there is no reason why these two reactions should not have equal heats of activation, but rates thousands of times different at the same temperature. There is then no doubt whatever, when it is found that all the known bimolecular reactions fit in in the same way, that the acquisition of the energy of activation is the all-important factor. The theory of the effect of temperature on reaction velocity shows the possession of this energy to be *necessary*, the kind of calculation which we have just been considering indicates that it may also be a *sufficient* condition for reaction.

When we attempt to go into the finer details of the reaction mechanism we can speak with less certainty. From the expression: *number of molecules entering into collision* $\times e^{-E/RT}$ we can calculate the absolute rate of reaction within a factor of two or three times. This is remarkable enough having regard to the fact that it is an *absolute* calculation, and that an essentially wrong theory would throw us out by many *powers of ten*. We can thus regard the χ of the previous section as adequately explained in terms of collisions. But to be able to answer the question as to whether literally *every* collision is effective, we should need experimental data much more accurate than we possess or are ever likely to possess. Some of the active molecules may rebound without suffering chemical change. We only know that this number is not large enough to interfere with the general concordance of the results with the theory we have been considering. The factors which limit the precision of our knowledge are (a) the approximate nature of the distribution law which gives rise to the simple exponential factor in the equation for the reaction rate (b) the approximate nature of our values for σ (c) the experimental difficulty of determining E with very great precision. E can usually be found to within 3 to 5 per cent; this leaves us in doubt about the value of $Z.e^{-E/RT}$ to the extent of a factor of two or three when the number of molecules reacting is of the order 10^{16} . A certain amount of not very profitable discussion has arisen on this point, i.e., whether "every" activated molecule reacts. It is not probable that we shall ever know.

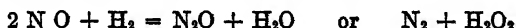
We can say, however, that we understand the nature of the factor χ in the case of bimolecular reactions.

TERMOLÉCULAR REACTIONS

Four termolecular gaseous reactions are known, all of which involve the participation of two molecules of nitric oxide. They are:



This last reaction takes place in stages thus



followed by the rapid decomposition of the nitrous oxide or hydrogen peroxide first produced. It is thus kinetically termolecular.

These reactions proceed in accordance with equations such as the following

$$-\frac{d[\text{NO}]}{dt} = k[\text{NO}]^2 [\text{O}_2] \quad \text{or} \quad -\frac{d[\text{NO}]}{dt} = k[\text{NO}]^2 [\text{H}_2]$$

The most natural supposition is that they depend upon a simultaneous collision between three molecules. It is however possible to assume that two molecules of nitric oxide first unite to form N_2O_2 and that there follows a bimolecular reaction between N_2O_2 and chlorine, bromine, oxygen or hydrogen as the case may be. If equilibrium between N_2O_2 and 2NO is established rapidly the concentration of N_2O_2 will always be proportional to $[\text{NO}]^2$, and since the rate of the bimolecular reaction will be proportional to $[\text{N}_2\text{O}_2] [\text{O}_2]$ for example, it will be proportional in turn to $[\text{NO}]^2 [\text{O}_2]$ and thus kinetically termolecular. This seems to be a rather artificial assumption; it is not really

¹⁶ Bodenstein (and Fr. Lindner), *Z. physik. Chem.*, **100**, 68 (1922).

¹⁷ Trautz, *Z. anorg. Chem.*, **88**, 285 (1914).

¹⁸ Trautz and Dalal, *Z. anorg. Chem.*, **102**, 149 (1918).

¹⁹ Hinshelwood and Green, *J. Chem. Soc.*, **129**, 780 (1926).

a necessary one, as we may see if we reflect on the peculiar nature of a ternary collision. In a binary collision there is a finite time during which two molecules are within a small enough distance of each other for a ternary collision to result should a third molecule arrive on the scene. This may be called the "duration of a collision." Now there is not a very great deal of difference from the kinetic point of view between two molecules of nitric oxide in a collision of finite duration, and the transitory formation of a molecule of N_2O_2 , or a so-called "complex" of two molecules of NO. There are, however, positive objections to the hypothesis of N_2O_2 formation which we shall be in a position to understand when we have considered the question of the temperature coefficient of termolecular reactions.

Bodenstein made the remarkable observation¹⁶ that the reaction $2\text{NO} + \text{O}_2 = 2\text{NO}_2$ has a very small but quite definitely negative temperature coefficient. Expressed in the usual way as the ratio of the velocity at $(t + 10)^\circ$ to the velocity at t° the coefficient changes from 0.912 in the neighborhood of 0° to 0.997 in the neighborhood of 350° . We should expect the temperature coefficient of a termolecular reaction to be much smaller than that of a bimolecular reaction for the following reason. A collision between three molecules is an event of great rarity compared with a binary collision. Hence if equal degrees of activation were required in two reactions, one termolecular and one bimolecular, the termolecular reaction would have to take place very much more slowly. Conversely, if the two reactions did in fact take place with the same speed at the same temperature, this would be because the termolecular reaction was associated with a much smaller energy of activation. Therefore, other things being equal, it would have a much smaller temperature coefficient. We can easily make a rough quantitative estimate of the difference to be expected. Bodenstein takes the ratio of the number of ternary collisions in a gas to the number of binary collisions as roughly equal to the ratio of the molecular diameter to the mean free path. This makes ternary collisions about 10^8 times less frequent at atmospheric pressure. The precise geometrical disposition of the molecules at the mo-

ment of impact is probably more important in a reaction like $2\text{NO} + \text{O}_2 = 2\text{NO}_2$ than in a simple bimolecular reaction like $2\text{HI} = \text{H}_2 + \text{I}_2$, so that the chance of a suitable termolecular encounter is probably about 10^4 times smaller than that of a suitable bimolecular encounter. Thus if a termolecular reaction is to attain the same rate as a bimolecular reaction at the same temperature, the energy of activation associated with it must be smaller by an amount ΔE such that $e^{-E/RT} \div e^{-(E+\Delta E)/RT} = 10^4$. At ordinary temperatures this means that the heat of activation would be about 6000 calories smaller, and at 1000° abs. about 15,000 to 20,000 calories smaller. It was pointed out in the last section that for bimolecular reactions the relation between heat of activation and absolute speed is fairly definitely known. A bimolecular reaction which could take place with measurable speed at the ordinary temperature would have a heat of activation of about 14,000 calories. Thus the heat of activation of a termolecular reaction which could occur rapidly enough to be measured at the ordinary temperature would not have to exceed about 8000 calories. The corresponding temperature coefficient would be quite small. The general discussion showed that the velocity constant of any reaction is a product of an exponential factor and another, being of the form $\chi e^{-E/RT}$. In bimolecular reactions χ involves the collision frequency, and thus increases in proportion to the square root of the absolute temperature. In termolecular reactions the term $e^{-E/RT}$ is such that a small positive temperature coefficient is to be expected. Thus the term χ , which depends on the frequency of ternary collisions must *diminish with temperature*, so that the velocity may in certain cases have a negative temperature coefficient, as for example in the reaction under discussion. Bodenstein suggests that this decrease is accounted for by the diminished "duration of collisions" at higher temperatures. The molecules move faster the higher the temperature; hence the smaller is the chance that two molecules shall remain close together long enough to be caught by a third molecule. The inversion of the temperature coefficient of the reaction velocity will of course only occur when the heat of activation is a small one, so that the "normal" temperature effect,

represented by the exponential factor, is easily masked. In the combination of nitric oxide with chlorine and bromine respectively the temperature coefficient has actually a small positive value but very little above unity. In a reaction which does not take place with appreciable speed except at quite high temperatures the effect of the diminishing collision frequency should no longer be noticeable, because the heat of activation should be quite large. The reaction between nitric oxide and hydrogen¹⁹ takes place in the neighborhood of 1100° abs. at a speed comparable with that of the reaction between nitric oxide and oxygen at the ordinary temperature. The heat of activation is 44,000 calories. A bimolecular reaction taking place with this same speed in the same region of temperature would be found to have a heat of activation of about 60,000 calories. It is obvious therefore that the relation between the two heats of activation is just about what would be expected from the relative chances of ternary and binary collisions. But quite precise calculations about termolecular collisions are difficult to make, so that it is not possible to analyze the matter in any more detail.

The discussion of these reactions becomes formally somewhat different if the view is adopted that they are really bimolecular reactions between N_2O_2 and a molecule of one of the other gases. The hypothetical bimolecular reactions must be assumed to have the normal temperature coefficients, but these are reduced or even inverted because the N_2O_2 is more and more dissociated at higher temperatures. It is very easy to show that the apparent heat of activation of the whole reaction is then equal to the difference between the heat of activation of the bimolecular reaction and the heat of dissociation of the double molecules of nitric oxide into single molecules. While formally this is quite satisfactory, Bodenstein points out that it necessitates the assumption of a quite considerable heat of formation of N_2O_2 , which therefore, in accordance with Nernst's theorem, should be fairly stable at low temperatures. Since no trace of N_2O_2 formation has ever been observed the objection is a serious one. It will be seen that a general understanding of the mechanism of termolecular reactions is possible, although numerical

calculations must at present deal with orders of magnitude rather than exact values. Still, within these limits the matter is quite clear. A very different state of affairs is found in dealing with unimolecular reactions.

UNIMOLECULAR REACTIONS

The thermal decomposition of nitrogen pentoxide,²⁰ and the thermal decomposition of gaseous acetone²¹ are homogeneous unimolecular reactions. The decomposition of sulphuryl chloride,²² although it takes place mainly on the walls in vessels of soda-glass, seems to be much less influenced by the walls in the case of vessels of Pyrex glass. The decomposition of phosphine,²³ at one time thought to be a homogeneous reaction, is mainly heterogeneous under the conditions where it can be measured. The claims that the transformation of cyclo-propane into propylene²⁴ becomes homogeneous at higher temperatures seem to rest upon a quite inadequate basis.

We shall confine our attention therefore to the decomposition of nitrogen pentoxide and to that of acetone. In the first reaction the nitrogen pentoxide decomposes into oxygen, nitrogen dioxide and tetroxide; in the second the acetone gives primarily carbon monoxide and ethane which suffers further decompositions. Nitrogen pentoxide decomposes at the ordinary temperature, acetone at a dull red heat.

The present position of the theory of unimolecular reactions may be outlined as follows:

1. Perrin²⁵ suggested that, since the rate of a unimolecular reaction is independent of the pressure of the gas, and it is thus possible to expand to an infinite volume without changing the probability that a molecule should be transformed, the process of chemical change cannot be determined in any way by molecular

²⁰ Daniels and Johnston, *J. Amer. Chem. Soc.*, **43**, 53 (1921).

²¹ Hinshelwood and Hutchison, *Proc. Roy. Soc., A*, **111**, 245 (1926).

²² D. F. Smith, *J. Amer. Chem. Soc.*, **47**, 1862 (1925).

²³ Trautz and Bhandarkar, *Z. anorg. Chem.*, **106**, 95 (1919). Hinshelwood and Topley, *J. Chem. Soc.*, 125, 393 (1924).

²⁴ Trautz and Winkler, *J. pr. Chem.*, **104**, 53 (1922).

²⁵ Perrin, *Ann. de Phys.*, [ix], **11**, 1 (1919).

collisions. This was the main support to the well-known "radiation theory" developed by W. C. McC. Lewis and by Perrin himself.

2. Two ways out of this situation have been proposed. Lindemann²⁶ pointed out that the ordinary experimental data relating to unimolecular reactions only proved that such reactions were independent of pressure over a certain range and did not necessarily justify extrapolation to infinite dilution. Independence of pressure over a wide, though not indefinitely extended range, can be accounted for without abandoning the hypothesis that activation is brought about by collision. It is only necessary to make the assumption that molecules gain and lose the energy of activation much more rapidly than they react. This could happen if the molecules had not only to receive the necessary energy but to be in a suitable internal phase before they could react, and if they got into this phase so rarely that they would probably lose the energy of activation they might gain in one collision by suffering another collision before reacting. By the distribution law there would be a constant fraction of the total number in possession of the energy of activation at any moment, whatever the pressure, and of these a constant fraction would react, this fraction being small enough to cause no appreciable change in the total concentration of active molecules. The reaction would thus be unimolecular over a considerable range of pressure. But at very low pressures the time between collisions would ultimately become so great that the loss of "active" molecules by chemical change could no longer be regarded as small in comparison with the rate of activation and de-activation. The supply could no longer be maintained, and the unimolecular velocity constant would decrease. The reaction would gradually assume the character of a bimolecular change. Christiansen and Kramers²⁷ propose what is known as the "chain mechanism." According to this the products of reaction, carrying away the original heat of activation plus any heat of reaction, are supposed to be able to activate by collision the next molecule of the

²⁶ Lindemann, *Trans. Faraday Soc.*, 17, 598 (1922).

²⁷ Christiansen and Kramers, *Z. physik. Chem.*, 104, 451 (1923).

reacting substance which they encounter. This process goes on indefinitely. The normal proportion of molecules which gain the energy of activation by collision is thus kept constant whatever the pressure, or however fast active molecules are removed by chemical reaction, because every such removal is forthwith replaced by a fresh molecule activated by the "hot" reaction products. Thus the chemical change can take place in accordance with the unimolecular law, although in essence bimolecular. According to this theory a marked retarding influence should be exerted by inert gases such as the products of reaction themselves, since these would remove the energy from the "nascent" molecules of product before this energy could be given to fresh molecules of the reactant. Christiansen and Kramers are obliged to make the arbitrary assumption that the reaction products do not give up their energy except to the right kind of molecule. An assumption of this sort seems to demand experimental support before it can be accepted.

We have thus the following possibilities:

Activation by collision	{ Lindemann mechanism Christiansen-Kramers mechanism
Activation by absorption of radiation	{ Simple radiation theory ²⁸ Extended radiation theory ²⁹

The simple radiation theory supposes that the molecule is activated by a quantum of monochromatic radiation, the frequency of which is given by E/Nh where E is the heat of activation, N is Avogadro's number and h is Planck's constant. A modification of the simple radiation theory supposes that several quanta are absorbed simultaneously or successively, while the extended radiation theory assumes that a whole continuous range of frequencies may be drawn upon for the supplying of the energy of activation.

These various possibilities will now be considered in relation to the experimental data for the decomposition of nitrogen pentoxide and of acetone.

²⁸ Perrin (25). W. C. McC. Lewis, *J. Chem. Soc.*, **109**, 796 (1916); **111**, 457 (1917); **113**, 471 (1918).

²⁹ Cf. Tolman, *J. Amer. Chem. Soc.*, **47**, 1524 (1925).

The simple radiation theory breaks down at once. It predicts that the decomposition of nitrogen pentoxide should depend upon the absorption of radiation in the short infra-red. Radiation of the calculated frequency is now known to be without influence on the rate of reaction. The calculated frequency for the acetone decomposition lies in the visible region, where acetone does not absorb.

There is no obvious direct test of the extended radiation theory. It will, however, require further consideration when the collision mechanisms have been examined.

The chain mechanism is highly improbable. Hunt and Daniels³⁰ showed that the presence of a very large excess of nitrogen did not influence the rate of decomposition of nitrogen pentoxide, even when the pressure of the pentoxide was very small. Hirst³¹ showed that argon had no effect on the rate of reaction. The rate of decomposition of acetone is not appreciably changed by the presence of nitrogen or of carbon monoxide, although experiments have not been made with very low pressures of the acetone.

Moreover it is not certain that the chain mechanism will work at all when the reaction is endothermic, as the unimolecular decomposition of N_2O_5 into N_2O_4 and O would be. It would however be unwise to reject the suggestion finally.

Lindemann's suggestion encounters still greater difficulties. In the first place, Hunt and Daniels found that the unimolecular character of the nitrogen pentoxide decomposition was preserved down to pressures of 0.01 mm. mercury, at least. Hirst and Rideal³² have confirmed this, and shown that the velocity constant at very low pressures actually tends to increase somewhat rather than to decrease. This last fact is important in that it removes the objection that the experiments were not made at pressures sufficiently low for the expected decrease to reveal itself. The increase is supposed to be due to the circumstance that at high pressures some de-activation of molecules takes

³⁰ Hunt and Daniels, *J. Amer. Chem. Soc.*, **47**, 1602 (1925).

³¹ Hirst, *J. Chem. Soc.*, 127, 657 (1925).

³² Hirst and Rideal, *Proc. Roy. Soc. A*, 109, 526 (1925).

place by collision with other molecules, and that this gets less at lower pressures.

A still more serious matter is that both in the decomposition of nitrogen pentoxide and in that of acetone the number of molecules which react is any way greater by very many times than the number which could be brought into the activated state by collision. The mechanism which is under discussion of course demands that the number of collisions should be not merely sufficient but much more than sufficient to keep up the supply of molecules with the necessary energy. In the case of the acetone decomposition the number of molecules reacting is about 10^5 times greater than the number which could be activated by collision.

It might be suggested that the calculations about the rate of activation by collision are founded upon false assumptions, and are quantitatively quite wrong. But the fact that analogous calculations yield such a coherent and satisfactory interpretation of bimolecular reactions makes this criticism seem rather improbable. A particularly striking contrast is found between the decomposition of gaseous acetone and that of acetaldehyde.¹⁰ Although these two reactions are chemically similar, and take place in the same region of temperature, the first is unimolecular and the second bimolecular, as shown by the influence of pressure. This means that the second definitely depends upon collisions. If the calculation of the maximum rate of activation by collision is wrong as applied to acetone, there is no particular reason why it should not give equally discordant results when applied to the bimolecular reaction of the aldehyde. Yet in this instance the maximum number of molecules which can be activated by collision comes out, as usual, to be almost equal to the number which do actually react.

On the whole, therefore, there seems to be no good ground at present for denying that unimolecular reactions are what they seem to be, namely changes in isolated molecules, not determined by collisions with other molecules. It must be admitted that views about this matter have veered round rather rapidly during the last few years and are still far from fixed.

As far as we can see at the moment it looks as if some gen-

eralized form of radiation theory is not excluded. Perhaps the view that the molecules interchange energy by any kind of thermal radiation which they are capable of absorbing or emitting is the most satisfactory. The whole range of isothermal radiation, with which any gas at constant temperature is in equilibrium, can then be invoked. This might quite possibly have been accepted without much discussion as a reasonable and innocuous suggestion if the controversies over the obviously inadequate special radiation theory had never been raised. The special radiation theory is destroyed by the criticism that exposure of the gas to a source of intense radiation of the appropriate wave length should cause an enormous acceleration of the reaction, which is not in fact observed. The view here suggested as possible, though by no means proven, is at least not open to this objection. The radiation is not supposed to be necessary in itself; it merely provides the means by which the Maxwell distribution of internal energy among the molecules is maintained in spite of the loss of activated molecules in reaction, and despite the inadequacy of collisions as a means of maintaining this distribution. It is essentially the isothermal radiation which is involved. This, from the very nature of the fact that it is in equilibrium with the gas, is presumably convertible into all the other forms of molecular energy. Thus we cannot attempt to increase the rate of reaction by irradiation from an external source, since in the attempt we should increase simultaneously all the different kinds of molecular energy, and thus raise the temperature of the whole gas. Acceleration of a reaction by increase of temperature is, of course, not a phenomenon which need cause us worry. Since the radiation need not be assumed to be more than an internal carrier of energy, the distribution law to be used in the equation for the velocity of reaction is that referring to the internal energy of the molecules, and is not governed directly by the nature of the radiation.

Another point is whether interchange of energy among molecules by radiation can occur at a sufficiently rapid rate. This has been discussed by several writers but the question cannot at the moment be said to have received any definite answer.

The whole matter must be regarded as open.

In the case of bimolecular reactions, the factor E/RT has approximately the same value for different systems at temperatures of equal reaction velocity. There are also strong indications of a similar proportionality of E and T in termolecular reactions. It is interesting to find that the same is true of unimolecular reactions. The heat of activation in the decomposition of nitrogen pentoxide is 24,700 calories. The velocity constant at 55° is 0.00150. The velocity constant of the acetone decomposition does not reach this value until 562° is reached, that is, an absolute temperature 2.55 times as great. We find, in keeping with this, that the heat of activation of the acetone is also much greater, namely 68,500 calories. The respective values of E/RT are 38.0 for nitrogen pentoxide and 41.4 for acetone. It is clear, therefore, that, in this example also, the heat of activation plays the principal rôle in determining the absolute rate of reaction; other factors must play a comparatively minor part.

A GENERAL RULE

On the basis of what has been said, an attempt may be made to formulate a general rule which has never before been expressly stated. It appears to be of fundamental importance, although of an approximate character. The velocity constant of a reaction can be expressed in the form $\chi \cdot e^{-E/RT}$. The rule is that among reactions of a given type the variations in χ are small compared with the variations in the exponential term, and that, therefore, the gaining of the energy of activation is the principal determining factor in reactions. There is an analogy to this in the process of vaporisation, where the chance of a molecule gaining enough energy to evaporate is $e^{-\lambda/RT}$, λ being the latent heat. The rate of evaporation at a given temperature is then $A \cdot e^{-\lambda/RT}$. Trouton's rule, which is roughly true, shows that for a given rate of evaporation λ/T is the same for most substances. Thus, variations in A from one substance to another are small compared with those of the exponential term. This again indicates that the acquirement of the energy is not only necessary, but sufficient, for evaporation to occur.

The rule about chemical reactions, which we have just discussed, is an approximation to the statement that the gaining of the energy of activation is not merely a necessary, but also a sufficient, condition for reaction to occur. This state of affairs is not exactly realized, but there is evidently an approach to it.

THE KIND OF ENERGY CONCERNED IN ACTIVATION

We have already discussed the question whether in unimolecular reactions the molecules are activated by collision or by radiation. The experimental evidence seems to show that they cannot be activated rapidly enough by collision. In polymolecular reactions collisions are necessary anyhow, whether they are directly concerned in the process of activation or not. Since the distribution law for all kinds of energy is approximately of the form $e^{-E/RT}$ it is easy to see that the number of collisions between molecules possessing already some-kind of energy in excess of E is nearly equal to the number of collisions in which the kinetic energy of translation exceeds this same amount. It is thus quite possible, or even probable, that the energy of activation in polymolecular reactions is, in the first instance, ordinary translational energy, which is converted into internal energy of the right kind at the moment of impact. But there is no reason why all forms of energy should not be operative at once, since there is a continual interchange between all the possible forms of thermal energy. For fuller discussion reference may be made to a paper of Tolman.²⁹

THE INFLUENCE OF INERT GASES AND OF MOISTURE ON THE RATE OF GASEOUS REACTIONS

In general this is small or negligible. Foreign gases, such as nitrogen, carbon dioxide or oxygen, do not accelerate or retard the simple bimolecular reactions such as the decomposition of nitrous oxide. Nor is the decomposition of nitrogen pentoxide affected by the presence of a large excess of foreign gases. Griffith and McKeown³³ came to the conclusion that the decomposi-

³³ Griffith and McKeown, *J. Chem. Soc.*, 127, 2086 (1925).

tion of ozone was somewhat retarded by oxygen and slightly accelerated by argon, nitrogen and helium. Unfortunately under the conditions of their experiments the reaction was partly homogeneous and partly heterogeneous, which makes the results a little inconclusive. (It is possible with suitable vessels to investigate the purely homogeneous reaction as Chapman and Clarke have shown.) But, from the fact that the velocity constants drift less when the reaction is accelerated by an inert gas than in the absence of the inert gas, the conclusion was drawn that the homogeneous change was the one accelerated. Griffith and McKeown suggest that the activated ozone molecules remain associated for a finite time during which they may collide with a molecule of the inert gas. Collision with oxygen favors the resolution of the complex into molecules of ozone once more, while collision with argon favors the resolution into the products of reaction. The magnitude of these effects is not great, but it is desirable that the matter should be investigated further.

Knowledge of the influence of inert gases is of particular interest in the case of reactions where two molecules unite to form a single one, or where two atoms unite to form a molecule, e.g., $\text{Br} + \text{Br} = \text{Br}_2$. Herzfeld³⁴ points out that when two atoms collide and form a molecule by an exothermic reaction, then the "nascent" molecule contains not only the original energy of the two atoms but also any heat liberated in the reaction. It should therefore, according to him, be incapable of continued existence unless it can give up this excess energy in a collision with some other molecule. Thus for the reaction $\text{A} + \text{B} = \text{AB}$ to complete itself there is needed not merely a binary collision of A and B but a ternary collision of A, B and a third molecule C. This argument does not apply to bimolecular reactions of the type $\text{A} + \text{B} = \text{C} + \text{D}$, because C and D fly apart after the collision in which they are formed, carrying with them the excess energy in the form of kinetic energy.³⁵ No reaction so far mentioned in this discussion is of the kind to which Herzfeld's theory is applicable. Nor are examples easy to find: reactions such as the union

³⁴ Herzfeld, *Z. Physik*, 8, 132 (1922).

³⁵ Born and Franck, *Ann. Phys.*, [iv], 76, 225 (1925).

of ethylene with chlorine³⁶ and with bromine³⁷ are surface reactions. The same is probably true of the union of hydrogen chloride and ammonia.³⁸ In these, therefore, there is naturally no difficulty about removing the heat of reaction from the "hot" newly formed molecules. The combination of phosphorus trichloride with chlorine³⁹ to form the pentachloride is, it is true, a homogeneous reaction, but its rate is much too great to be measured.

Indeed, the only reaction which is suitable for the testing of Herzfeld's theory is the combination of bromine atoms to form molecules. The rate of this was determined by an indirect method by Bodenstein and Lütkemeyer⁴⁰ and by Bodenstein and Müller.⁴¹ It was not influenced by inert gases in the sense to be expected; the fraction of the total number of collisions between bromine atoms which leads to combination was found to be rather a small one, but it was independent of the total pressure of the gases present in the system.

It is, of course, now recognized that, since molecules possess more than a single degree of freedom, it is quite possible for them to contain energy considerably in excess of the energy of decomposition into their atoms. If, therefore, the heat of reaction were shared among several degrees of freedom there is no reason why the molecule should be incapable of continuing to exist when once formed. Since, however, the vibrational and rotational energy are quantised, it is improbable that the amount of energy to be shared out will be an exact number of quanta. Hence the sharing will be impossible unless the difference between the total energy and some exact number of quanta can be disposed of. In reactions where two or more molecules of product are formed the translational energy of the rebounding molecules provides a means of adjustment. When one molecule only is formed the

³⁶ Norrish and Jones, *J. Chem. Soc.*, 129, 55 (1926).

³⁷ Stewart and Edlund, *J. Amer. Chem. Soc.*, 45, 1014 (1923).

³⁸ Unpublished observations of R. E. Burk in Oxford.

³⁹ H. A. Taylor, *J. Physical Chem.*, 28, 510 (1924).

⁴⁰ Bodenstein and Lütkemeyer, *Z. physik. Chem.*, 114, 208 (1924).

⁴¹ Bodenstein and Müller, *Z. Elektrochem.*, 30, 416 (1924).

excess would have to be radiated away. The radiation need only be a small quantum of some appropriate quite low infra-red frequency, which would not be noticed in an ordinary experiment. But little is known about this.

Of the chemical changes which have been investigated, homogeneous gas reactions do not constitute a very large fraction. Hence it is not always safe to apply to them conclusions which have been reached in quite different fields. The question has sometimes been raised whether it is profitable to attempt the interpretation, in terms of the kinetic theory, of reactions which, it is alleged, would not go at all if the gases were completely dried. This question is based upon a misconception. Most of the reactions investigated by Baker and others, and which have been found to be inhibited by intensive drying are either reactions between substances in different phases, e.g., the action of sulphur trioxide on barium oxide, or are surface reactions—the combination of ammonia and hydrogen chloride is probably an example of this kind. The photochemical union of hydrogen and chlorine is inhibited by drying,⁴² but this is a special case of a very peculiar kind, which presents abnormal features in nearly every possible respect. In none of the simple homogeneous gas reactions of definite order, of which the molecular statistics have been worked out, has the inhibition by drying been demonstrated, or even claimed. Nor would such an effect be intelligible. A typical instance of the behaviour of a system where reaction is stopped by drying is provided by the union of ammonia and hydrogen chloride. Unless quite exceptional precautions are taken to dry the containing vessels, the reaction takes place with immeasurable rapidity. If the walls are completely dried, the reaction does not take place at all. There is no regular proportionality between water content and reaction velocity, as there would be if a definite ternary interaction between NH_3 , H_2O and HCl occurred. On the other hand, in a heterogeneous reaction this behavior is quite understandable;⁴³ if there is sufficient water to form a uni-

⁴² Coehn and Jung, *Z. physik. Chem.*, **110**, 705 (1924).

⁴³ Bowen, *J. Chem. Soc.*, **125**, 1235 (1924). Norrish, *Faraday Society. Discussion on Photochemical Reactions in Liquids and Gases.* (1925), page 575.

molecular layer over the surface, the full effect is exerted, and any excess beyond this minute trace has no further influence.

The explosion of carbon monoxide and hydrogen is presumably a homogeneous change, even if rather ill-adapted for measurements relating to the kinetics of the reaction. In this example, however, there is, just as we should expect, a regular proportionality over an extended range between water content and the speed of the explosion wave. The water here plays a definite stoichiometric part in the series of changes, the first of which is probably $\text{CO} + \text{H}_2\text{O} = \text{CO}_2 + \text{H}_2$.

We must, in short, regard purely negative criticisms of the results of kinetic studies of gas reactions as uninformed.

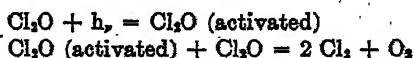
PHOTOCHEMICAL REACTIONS IN GASES

Light is absorbed and emitted by molecules in quanta the size of which is determined by the well-known relation— $\text{energy} = h\nu$. Thus illumination with monochromatic light provides a means for supplying to the molecules accurately known amounts of energy. It might therefore be expected that a simple relationship would exist between the frequency of the photochemically active light and the heat of activation. Any hope of discovering more about the mechanism of chemical reactions in this way is, however, disappointed. The reason is not difficult to see, and can be explained best with a simple illustration. Let us consider the chlorine molecule. In this two atoms are united by what is called a non-polar link, which probably involves electronic orbits common to both nuclei. Each atom has also its own independent electrons. Stimulation of the molecule by light in the visible region of the spectrum causes these latter electrons to move to higher quantum orbits. Much energy may be absorbed by one or other of the two atoms in this way before the link between them is affected and the molecule decomposed. In fact, the energy taken up may be much greater than the energy of dissociation. Photochemical stimulation puts the energy in the wrong place; it is an extravagant method of molecular activation. We find, quite in keeping with this idea, that the quanta

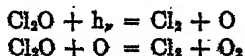
involved in photochemical changes are in general much greater than ordinary chemical heats of activation.⁴⁴

Nevertheless the study of photochemical changes has led to the discovery of certain facts of fundamental importance to chemical kinetics generally. In most cases these reactions conform more or less to the Einstein law of photochemical equivalence. The basis of this law is best stated as follows: (a) Light is in the nature of things absorbed by the molecules in quanta of magnitude $h\nu$. (b) The quantum of light corresponding to the visible region of the spectrum is a large quantity of energy for one molecule to possess, and, if a molecule does not react at once when the light is absorbed, it is almost certain to lose the energy again in a collision before it has the chance to absorb a second quantum. Thus processes in which molecules absorb more than one quantum of visible light are seldom or never found. (c) The light quantum being an amount of energy very many times greater than the average energy of a molecule at ordinary temperatures, it is not likely that the effectiveness of the light in bringing about chemical change will be much influenced by minor variations in the internal state of the molecule. Thus if the light is capable at all of provoking the reaction, it is probable that each quantum will be effective. This is the Einstein law which states that one molecule is caused to react for each quantum of the active light which is absorbed.

In the decomposition of hydrogen bromide and of hydrogen iodide in the gaseous state Warburg⁴⁵ found that two molecules are decomposed for each quantum absorbed. Bowen⁴⁶ found the same to be true of the decomposition of chlorine monoxide. This is explained by assuming the mechanism:



OR

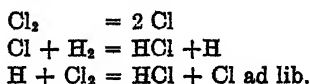


⁴⁴ Bowen, *ibid.*, page 543.

⁴⁵ See collected summary by Allmand, *ibid.*, p. 444.

The formation and decomposition of ozone roughly conform to the Einstein law, as also do the combination of chlorine and sulfur dioxide and the bromination of C_6H_{12} .⁴⁵ Only one molecule in four of ammonia appears to decompose. This result however may not be very exact.

On the other hand, many molecules of phosgene are formed from carbon monoxide and chlorine for a single quantum, and hundreds of thousands of molecules of hydrogen and chlorine combine for each quantum of light. Reactions of this kind have to be interpreted by what is called the "chain mechanism." An example of the sort of way in which this works is the following:



This is illustrative merely. In the hydrogen-chlorine reaction water intervenes also in the reaction chains. In general, there is a primary photochemical reaction followed by a series of purely thermal changes. Weigert and Kellermann⁴⁶ have found direct evidence of the existence of these chains by examining cinematographically the condition of the gas after momentary illumination with a spark.

It is a matter open to question, however, whether the reaction chains in the hydrogen-chlorine combination are not partly subject to the influence of the walls of the vessel. Opinion on this matter is divided, and decisive experiments have not been made.

An interesting region of transition between photochemical and thermal reactions is opened up by the experiment of Cario and Franck,⁴⁷ in which a mixture of hydrogen and mercury vapor was exposed to the light of the mercury line 2536.7 Å. The mercury atoms, excited by the light, were able on collision to resolve hydrogen molecules into atoms—or at least to convert them into a chemically active form. A number of other experiments of this kind were made by Taylor and Marshall,⁴⁸ who activated

⁴⁵ Weigert and Kellermann, *Z. physik. Chem.*, **107**, 1, (1923).

⁴⁷ Cario and Franck, *Z. Physik*, **21**, 161 (1922).

⁴⁸ Taylor and Marshall, *J. Physical Chem.*, **29**, 1140 (1925).

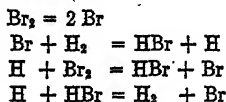
the hydrogen in this way and caused it to react with ethylene, carbon monoxide, oxygen and nitrous oxide in the cold. According to Hirst and Rideal,⁴⁹ however, these reactions only take place when liquid mercury is present and its surface is illuminated. The reactions according to this are heterogeneous. In any case the possibility of mercury hydride formation must not be ignored.

MISCELLANEOUS REACTIONS

An interesting, if specialised, question is that of the part played by free atoms in chemical kinetics. The rate of decomposition of carbonyl chloride⁵⁰ in the region of 700° is proportional to the square root of the concentration of the chlorine present. This suggests that the decomposition depends upon a collision of a carbonyl chloride molecule with a chlorine atom, the concentration of atomic chlorine being proportional to the square root of that of the molecular chlorine, since $[Cl_2] = K [Cl]^2$.

Norrish and Rideal⁵¹ suppose the interaction of hydrogen and sulfur in the gaseous state to take place between hydrogen molecules and sulfur atoms, formed by the dissociation of more complex molecules in the sulfur vapor.

Bromine atoms appear to intervene in the very complicated reaction mechanism by which hydrogen and bromine combine.⁵² The following stages are supposed to be involved:



This mechanism leads to the equation

$$\frac{d[2 H Br]}{dt} = \frac{k[H_2] \sqrt{[Br_2]}}{m + \frac{[2 H Br]}{[Br_2]}}$$

⁴⁹ Hirst and Rideal, *Nature*, **116**, 899 (1925).

⁵⁰ Christiansen, *Z. physik. Chem.*, **103**, 99 (1922). Bodenstein and Plaut, *ibid.*, **110**, 399 (1924).

⁵¹ Norrish and Rideal, *J. Chem. Soc.*, **123**, 696, 1689, 3202 (1923).

⁵² Bodenstein and Lind, *Z. physik. Chem.*, **57**, 168 (1906).

where m is a constant. This equation was established empirically by Bodenstein and Lind before the theory of the process was proposed.

Reactions of this kind depending upon a quite specialized reaction mechanism are usually too complicated to be analyzed completely into their separate steps in such a way that the heat of activation of each can be found.

DIRECTIVE INFLUENCE IN THE BENZENE RING

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An admirable review² by A. F. Holleman appeared two years ago upon the subject, "Some Factors Influencing Substitution in the Benzene Ring." The scope of the present paper is somewhat more limited. It is the intention to avoid unnecessary repetition and to discuss the subject from slightly different view points. The older theories, which are considered in all organic text books, will not be discussed except as related to more recent publications.

The term "directive influence" will be used to indicate the effect which a substituent already present in benzene exerts upon the position assumed by an entering group. The orienting effects of other factors such as temperature, solvent, catalysts, and the identity of the entering group are usually comparatively slight, except as steric influences are involved. They have been discussed adequately in Holleman's paper, and will not be considered here.

It is generally agreed that directive influence is a manifestation of the *relative* rates of substitution in the several available positions of a benzene nucleus. It is for this reason that conditions external to the molecule usually have little effect, since they are likely to influence the speed of each reaction to nearly the same extent. Indeed this idea has been extended to directive influence itself³ for certain types of substitution. But the relative rates themselves must be due to some strained condition in the molecule. I agree with Holleman that a study of the

¹ The author's own research in this field was conducted partly as a graduate student at Yale University, and partly as a National Research Fellow at the Massachusetts Institute of Technology.

² Holleman, *This Journal* 1, 187-230 (1924).

³ Francis, *J. Am. Chem. Soc.* 47, 2588 (1925).

rates of these reactions is the best method of attack; but disagree with him in his rejection⁴ of such ideas as the strength of Flürscheim's bonds⁵ on the basis that they are vague ideas incapable of measurement. The reaction velocities appear to be the most accurate means of determining the relative strength of these bonds.

FUNDAMENTAL CHARACTER OF DIRECTIVE INFLUENCE

It has been asserted that directive influence is a phenomenon which *merely* concerns rates of reaction. In support of this Lewis has stated, "There is no evidence at present that the compound produced (under the influence of an *ortho* orienting group) is any more stable than one which would be produced by the corresponding substitution in the *meta*."⁶ He is undoubtedly correct, since, using stability in a thermodynamic sense, the most stable isomer, that is, the one with the least free energy, cannot have been produced in *both* of the following two reactions: If nitrobenzene is chlorinated, the product is mainly *m*-nitrochlorobenzene; while the nitration of chlorobenzene gives chiefly the *para* isomer. Furthermore, in no case is there a true equilibrium between the isomers.

There is some evidence, however, that directive influence is not a phenomenon which is displayed only at the moment of reaction. It seems likely that in most cases the least reactive isomer is formed most rapidly, provided the question of reactivity be limited to the lability of the substituent which has just entered. This is evidently true in the chlorination of nitrobenzene, since *o*- and *p*-nitrochlorobenzenes are readily hydrolyzed to the corresponding nitrophenols, while the *meta* isomer resists hydrolysis. In the nitration of chlorobenzene the issue is the lability of the nitro group; and though there is no direct evidence, the data which follow make it probable that it would be displaced more readily from *m*-nitrochlorobenzene than from the *ortho* and *para* isomers.

⁴ Holleman, *J. prakt. Chem.* 74, 157 (1906).

⁵ Flürscheim, *ibid.* 66, 321 (1902); 71, 497 (1905); *Ber.* 39, 2015 (1906).

⁶ Lewis, Valence and the Structure of Atoms and Molecules. The Chemical Catalog Co. 1923. p. 146.

Fortunately there is an abundance of material upon the reactivity of $-\text{NO}_2$ or $-\text{Cl}$ in the higher derivatives of benzene, using sodium methylate as a reagent. *m*-Dichlorobenzene, for example, is hydrolyzed more readily than its isomers.⁷ The other results are summarized in table 1. The number of the substituent displaced by $-\text{OCH}_3$ is printed in bold type.

TABLE 1
Displacement of substituents with sodium methylate

Trichlorobenzenes ¹	1 2 3; 1 2 4; 1 3 5
Tetrachlorobenzenes ²	1 2 3 4; 1 2 3 5; 1 2 4 5
Pentachlorobenzenes ²	1 2 3 4 5
Nitrodichlorobenzenes ³ ($-\text{NO}_2 = 1$).....	1 2 3; 1 2 4; 1 2 5; 1 2 6; 1 3 4; 1 3 5
Nitrotrichlorobenzenes ⁴ ($-\text{NO}_2 = 1$).....	1 2 3 4; 1 2 3 5; 1 2 3 6; 1 2 4 5; 1 2 4 6; 1 3 4 5
Chlorodinitrobenzenes ⁵ ($-\text{Cl} = 1$).....	1 2 3; 1 2 4; 1 2 5; 1 2 6; 1 3 4; 1 3 5
Chlorotrinitrobenzenes ⁵ ($-\text{Cl} = 1$).....	1 2 3 4; 1 2 3 5; 1 2 3 6 (unknown); 1 2 4 5; 1 2 4 6; 1 3 4 5
Dinitrodichlorobenzenes ⁶ (First two numbers = $-\text{NO}_2$).....	1 2 3 4; 1 2 3 5; 1 2 3 6; 1 2 4 5; 1 3 2 4; 1 3 2 5; 1 3 4 5; 1 3 4 6; 1 4 2 3; 1 4 2 5; 1 4 2 6
Dinitrotoluenes ⁷ ($-\text{Me} = 1$).....	1 2 3; 1 3 4; 1 2 5

¹ Holleman, *Rec. trav. chim.* **37**, 195 (1918).

² Holleman, *ibid.* **39**, 736 (1920).

³ Holleman, *ibid.* **35**, 1 (1916).

⁴ Holleman and Hoeften, *ibid.* **40**, 67 (1921).

⁵ van de Vliet, *ibid.* **43**, 606 (1924).

⁶ Holleman, *ibid.* **39**, 435 (1920).

⁷ Kerner and Parkin, *J. Chem. Soc.* **117**, 852 (1920).

In general that substituent is labile which is most out of position with respect to the directive influence of the other substituents, the influence of $-\text{NO}_2$ for this generalization being greater than that of $-\text{Cl}$. In fact Obermiller⁸ used this phenomenon as a test of directive influence, and so concluded that $-\text{NO}_2$ possesses greater power of orientation than $-\text{OH}$. This

⁷ Holleman, *Rec. trav. chim.* **35**, 1 (1916).

⁸ Obermiller, *J. prakt. Chem.* **89**, 70 (1914); *Z. angew. Chem.* **27**, 37 (1914).

cannot be true since in *m*-nitrophenol further substitution is always in the 2, 4, and 6 positions under the influence of $-OH$, and never in 5, where the nitro group would tend to place it. The above relation in the nitrochlorobenzenes holds also in a quantitative sense, rates of displacement of groups, as determined by Holleman⁹ depending upon the same factors. The relation was pointed out by Kerner and Parkin,¹⁰ and by Fraser and Humphries¹¹ who stated, "A group which causes *substitution* in the *meta* position will favor *replacement* in the *ortho*-*para* positions, and conversely." They also gave a mechanism for the displacement, which explains some of the exceptions.

Evidence for the fundamental character of directive influence is found also in a recent paper¹² in which there have been pointed out approximate relations of directive influence with melting temperatures and heats of fusion of isomeric derivatives of benzene. According to this, the *meta* isomer has the *lowest* entropy of fusion (heat of fusion divided by the absolute melting temperature) when the two groups are like in their directive influence, and the highest when they are unlike. Similarly in most of the commoner systems the *meta* isomer has the lowest melting temperature when the groups are like, and the *ortho* isomer has the lowest when they are unlike. These observations were made reasonable by the following considerations which, it is admitted, do not constitute a rigorous train of reasoning. When the two groups are like in their influence, there is coöperation between them in favoring further substitution in the *meta* isomer, and opposition in the *ortho* and *para* isomers. When they are unlike, the case is reversed, and the *ortho* isomer is the most reactive. It is thus not illogical to suppose that the most reactive isomer is the one most easily melted,¹³ that is, it is the one that has the lowest melting temperature and the lowest entropy of fusion. The implication is that in reactions suffi-

⁹ Holleman, *Rec. trav. chim.* **42**, 373-9 (1923).

¹⁰ Kerner and Parkin, *J. Chem. Soc.* **117**, 852 (1920).

¹¹ Fraser and Humphries, *Chem. News* **126**, 258 (1923).

¹² Francis, Andrews, and Johnston, *J. Am. Chem. Soc.* **48**, 1624 (1926).

¹³ The original (p. 1630) must be consulted for an explanation of this statement.

ciently similar, there is some parallelism between the rate of reaction and the change of free energy. This is supported by the comparative constancy of directive influence (Holleman's "conservation of the substitution type"¹²) under wide variations of external conditions. Prins has stated,¹⁴ "We are convinced that at least for analogous reactions the velocity is determined by the atom energy."

The melting temperature relation was observed also by Pastak,¹⁵ but he discussed it in quite a different manner. He considered that the *meta* position was "normal" for like substituents and the *ortho* and *para* positions normal for unlike groups. This is logical for the products of nitration, but for halogenation the reverse would be expected. His "normal" isomers are the more reactive ones, and therefore, *less* stable rather than "more stable."

DISTINCTION BETWEEN ORTHO-PARA AND META ORIENTING GROUPS

The several rules which have been proposed for classifying the substituents according to directive influence, namely those of Hübner, Nölting, Körner, Armstrong, Brown and Gibson, and Vorländer, are well known, and have been discussed by Holleman. None of them holds for more than four-fifths of the known cases. But some recent contributions deserve attention.

Miss Hoffert¹⁶ observed that if the numbers of valence electrons of all the atoms of a substituent be added together, the sum is one less than a multiple of eight for *ortho-para* controlling groups, which are therefore negative, and one more than a multiple of eight for *meta* directing or positive groups. Thus the sum is 7 for the halogens, $-\text{OH}$, $-\text{NH}_2$, $-\text{CH}_3$, 15 for $-\text{CNS}$, $-\text{CNO}$, and 23 for $-\text{CH}_2\text{COOH}$, $-\text{NHCOCH}_3$; and it is 9 for $-\text{CN}$, 17 for $-\text{COOH}$, $-\text{NO}_2$, $-\text{COCH}_3$ and 25 for $-\text{SO}_3\text{H}$ and CCl_3 . In $-\text{CH}=\text{CHCOOH}$ four electrons are shared in the double bond and should be subtracted, making the sum 23 in-

¹² Prins, *Rec. trav. chim.* **44**, 169 (1925).

¹⁵ Pastak, *J. chim. phys.* **22**, 43, 264 (1925).

¹⁶ Hoffert, *J. Soc. Chem. Ind.* **42**, 348 (1923).

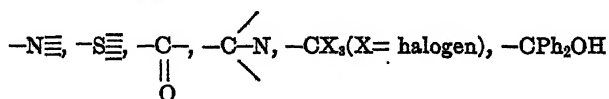
stead of 27, so that it is *ortho-para* directing. Other groups as $-\text{CHO}$ with 11, and $-\text{CH}_2\text{Cl}$, $-\text{CH}_2\text{OH}$, and $-\text{OCH}_3$ with 13 valence electrons each, have intermediate values and should give mixed derivatives.

Unfortunately there are exceptions to this ingenious rule. $-\text{OCH}_3$ is really a very strong *ortho-para* directing group; and according to the rule, $-\text{COOMe}$ should be an *ortho-para*, and butyl and $-\text{OPr}$ *meta* directing groups, whereas actually the reverse is true. Moreover, not four but two units should be subtracted for a double bond, because although two electrons are shared in a single bond, the rule does not call for a corresponding subtraction of two units. If a carbon-carbon double bond should receive exceptional treatment, so should other double bonds as in $-\text{NO}_2$ or $-\text{COOH}$.

Olivier¹⁷ has shown a parallelism between directive influence and ease of hydrolysis of benzyl chlorides. He stated that if the *para* isomer of $\text{XC}_6\text{H}_4\text{CH}_2\text{Cl}$ is most easily hydrolyzed, X is an *ortho-para* directing group; while if the *meta* isomer is most readily hydrolyzed, it is a *meta* directing group. This rule is most interesting from a theoretical standpoint as well as in ascertaining directive influence. It would be of great importance to know if any exceptions can be found. The work of Norris and his students upon derivatives of diphenylchloromethane¹⁸ is in substantial agreement with Olivier's results. Olivier's observations will be discussed later.

I have classified the groups empirically as follows:

Meta directing groups:



Ortho-Para directing groups:

Halogens, $-\text{N}=\text{}$, $-\text{O}-$, $-\text{S}-$, $-\text{C}\equiv$ (except those included in the *meta* category)

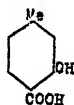
¹⁷ Olivier, *Rec. trav. chim.* **42**, 775 (1923).

¹⁸ Norris, Private communication.

in which the left hand valence is attached directly to a benzene ring, and the right hand valences to any atom or atoms. I know of no exceptions.

CUMULATIVE EFFECT OF TWO OR MORE SUBSTITUENTS

Holleman has given an extended discussion² of the combined effect of two or more groups upon further substitution. Indeed this has been involved in the bulk of his voluminous researches. It is generally agreed that each substituent continues to exercise its own peculiar influence, but just how the combined effect should be calculated is not so clear. Holleman has devised two methods, the *product rule* and the *sum rule*, both of which are useful, though somewhat empirical, and not altogether satisfactory. In my own research I have shown the cumulative effect of substituents in a different manner,¹⁹ by estimating the rate of bromination in aqueous solution of higher benzene derivatives, such as *m*-cresotinic acid.



This is a derivative of *m*-cresol, and also of salicylic acid. By multiplying together the velocity constants of bromination of these two, 100 and 0.4 (compared to aniline as unity), and dividing by that of phenol, 21 (so as not to count the effect of $-OH$ twice), the value 1.9 was obtained. The observed value was 2.1, which is within experimental error. Similarly 2,6-dibromo-aniline has a constant, 25, which is the square of that of *o*-bromoaniline, 5. It must be admitted that the agreement is not always so good.

ANALYTICAL METHODS

Any study of the positions assumed by substituents presupposes an accurate analytical method for the resulting mixtures of

¹⁹ Francis, *J. Am. Chem. Soc.* 48, 1631 (1926).

isomers. Obviously no analysis for a single element would be of any use for this purpose. This does not mean, however, that there are no chemical methods which will avail. Selective displacement of groups²⁰ has been used in special cases to distinguish between isomers; and the analysis of mixtures of the xylenes is best accomplished by selective sulfonation.²¹ Dimroth has employed the fact that hydrazine hydrate reacts with *o*- and *p*-nitrobromobenzenes but not with the *meta* isomer.²² Presumably this differentiation would apply to the other nitrohalogenbenzenes.

The most general chemical method is that of aqueous bromination.²³ This applies to practically all phenols and amino and nitro compounds, which include a majority of the derivatives of practical importance. The method depends upon the fact that all positions *ortho* or *para* to an $-NH_2$ or $-OH$ group, and not already filled, are substituted by bromine. Thus a *meta* compound requires three equivalents of bromine, while an *ortho* or *para* isomer requires only two, one of the positions being already filled. Nitro compounds are first reduced quantitatively to the corresponding amino compounds with a definite amount of standard titanous chloride, and then titrated in the same solution with bromide-bromate reagent. The method does not distinguish between *ortho* and *para* isomers, but in studying directive influence this distinction is not usually so important as that of the *meta* from the *ortho* and *para* compounds; and if a complete ternary analysis is required, a combination of methods is most efficient, since the *para* isomer is usually the one most easily estimated by physical means. The bromination method does not require the extensive preliminary research upon each system of isomers which is involved in other analytical methods. A sample of 0.1 gram is sufficient for a determination, and the substance need not be free from foreign impurities. It is also

²⁰ Table 1, Holleman, *Rec. trav. chim.* **23**, 253 (1904); **24**, 143 (1905); Wagner, *Ber.* **7**, 76 (1874).

²¹ Levinstein, *J. Soc. Chem. Ind.* **3**, 77 (1884).

²² Dimroth, *Ann.* **446**, 148 (1925).

²³ Francis and Hill, *J. Am. Chem. Soc.* **46**, 2498 (1924).

frequently applicable to the higher derivatives of benzene, which may require three, two, one, or no equivalents of bromine. An analysis can be completed in from ten to thirty minutes.

Mixtures of isomeric derivatives of benzene have usually been analyzed by physical means, of which the favorite is that employing freezing curves.²⁴ Isomers usually form ideal solutions with each other so that the freezing point of one isomer is depressed the same amount by equal percentages of either of the other two. This facilitates the construction of the ternary freezing diagram. After the isomer which crystallizes first from the melt has been identified, the crystallization temperature, corrected for supercooling, gives at once the percentage of that isomer. Another isomer is estimated by adding to the mixture a sufficient amount of that one to cause it to crystallize first. The operation is tedious and the effect of foreign impurities, even water, is difficult to estimate. These impurities cannot easily be removed without changing the relative amounts of the isomers. Moreover, the method is not as general as is commonly supposed, since the isomers of some systems, for example, the cresols, form compounds with each other. The technique has been improved recently by Johnston and his students.²⁵

Other physical methods which have been used are those depending upon differences in solubility,²⁶ critical solution temperature,²⁷ and density²⁸ of the isomers. A method employing absorption spectra²⁹ has been proposed for this differentiation.

METHODS OF STUDY

A complete understanding of the subject will be attained only by the proper interpretation of an enormous mass of experimental

²⁴ Holleman and others, *Rec. trav. chim.* 28, 411 (1909); 30, 55, 365 (1911); 31, 244 (1912); 33, 1 (1914); *Ber.* 44, 704 (1911).

²⁵ Johnston, Kohman, and Andrews, *J. Phys. Chem.* 29, 882, 914, 1041, 1048, 1317 (1925).

²⁶ Holleman and others, *Rec. trav. chim.* 18, 267 (1899); 19, 89, 367 (1900); *Z. physik. Chem.* 31, 79 (1899).

²⁷ Hoeflake, *Rec. trav. chim.* 36, 36 (1917).

²⁸ Lunge, *Chem. Ind.* 8, 74 (1885). Compare Allen's "Commercial Organic Analysis." Blakiston, 1914, vol. VI, p. 70.

²⁹ Courtet and Dondelinger, *Bull. soc. chem.* 37, 115 (1925).

data of which the present supply is entirely inadequate. Authorities are not even agreed as to the order in strength of directive influence of some of the commonest substituents, for example, methyl and the halogens,³⁰ the halogens among themselves,³¹ $-\text{NO}_2$ and $-\text{SO}_3\text{H}$.³² A partial explanation of this disagreement will be discussed later.

The obvious method of attack is to treat various compounds with a substituting agent, usually nitric acid, sulfuric acid, or a halogen, and to analyze the resulting mixture of isomers. Nitric acid has been preferred because of the low temperature of reaction, the ease with which substitution can be made to take place in steps, as desired, and the comparative ease of analysis. The directive strengths of two substituents are compared by choosing a disubstituted benzene, for example, *p*-chlorotoluene, in which the influences are opposed; that is the methyl group would tend to place a new substituent in the 2 position, and chlorine in the 3 position. But since chlorine directs mainly to the *para* position (which is blocked) and methyl preferably to the *ortho* position, the latter has an advantage in this case; and a true comparison can be obtained only by studying also the substitution of *o*- and *m*-chlorotoluene and mixtures of toluene and chlorobenzene, all of which has been done by Wibaut.³³

Another method is to compare the *rates* of substitution of several compounds in a similar way. This requires careful control of temperature, catalysts or impurities, and medium. It is not certain what conditions in various reactions are comparable, as Holleman himself has observed. He has initiated the bulk of the research upon both the methods mentioned.

In entering this field, I was led to investigate directive influence by studying the relative rates of bromination of various amino and phenolic compounds in aqueous solution. This method had

³⁰ Compare Holleman, ref. 2, p. 202 with Challenger and Collins, *J. Chem. Soc.* 125, 1377 (1924).

³¹ Compare Holleman, ref. 2, p. 202, with Holleman, "Die direkte Einführung von Substituenten in den Benzolkern," Veit and Co., Leipzig, 1910, pp. 469-70.

³² Compare Holleman, ref. 2, p. 202, with Obermiller, ref. 8.

³³ Wibaut, *Rec. trav. chim.* 32, 244 (1913); 31, 241 (1915); *Chem. Ztg.* 35, 762 (1914).

been entirely neglected because of the evident obstacles that all the reactions are "instantaneous," and that they cannot usually be limited to a single equivalent of substitution. On the other hand, the reactions proceed quantitatively in the cold, and the products in most cases can be separated easily from the reaction mixture.

On the supposition that the rates are not identical, "competition" experiments were devised³⁴ in which two organic compounds in acidified aqueous solution were treated with an insufficient amount of bromine, in the form of potassium bromide and potassium bromate, which with acid generate bromine slowly. The two organic compounds thus compete for the bromine as it is generated, and the relative rates of the two bromination reactions can be estimated from the amount of either of the bromination products. The experiments can be so chosen that the analysis for this product is usually simple, generally filtering, washing, and drying a precipitate. The whole technique of these experiments thus becomes very simple, but the calculation of the results is complex. This is due to the fact that the substitution of two or three equivalents of bromine usually takes place in each competitor, and these several reactions are sometimes consecutive and sometimes simultaneous and sometimes partly both. In making the calculations "partial bromination curves"^{3,34} were used. These are derived from similar experiments upon a single compound with insufficient bromine, and show the relative yield of highest brominated product plotted against the relative amount of bromine added, compared in each case with the theoretical. Fortunately a comparatively small number of curves sufficed for all the compounds, since with a few exceptions, all *para* amino compounds follow one curve, all *ortho* amino compounds another, and all *meta* amino compounds a third, which is the same as that for aniline; and in most phenolic compounds the several substitutions were found to be successive and distinct. For details in choice of competitors, procedure, and calculations the reader is referred to the original publications.³⁵

³⁴ Francis, Hill, and Johnston, *J. Am. Chem. Soc.* **47**, 2211 (1925).

³⁵ Ref. 34, pp. 2222-4 and ref. 3, pp. 2589-90.

This method of experimentation was found to have certain advantages not entirely anticipated. One is that in the comparison of two reactions it is certain that the conditions are alike because they are identical. It is not necessary to control accurately the temperature or the concentration because it has been shown²⁶ that the *relative* rate of the two reactions is practically independent of these factors; and it is only the relative rate that is obtained, or is required. It is true that even the relative rate is affected considerably by the medium, for example the concentration of acid or alcohol (frequently necessary to maintain the substance in solution); but with suitable precautions no serious difficulty need arise. Another advantage in the method is the comparatively rapid progress that is possible because of the simplicity of experiments; and this is not negligible when it is considered that not by a few experiments, however accurate or refined, but only by thousands of them, can the problem of directive influence be solved. This does not mean that any care in manipulation was sacrificed to speed.

The conclusions of this work will be considered in connection with the corresponding ones of other investigators.

MECHANISM OF SUBSTITUTION IN THE BENZENE RING

The mechanism of substitution has been occasion of much controversy which still continues. This is due to the comparative lack of direct experimental evidence for any mechanism, and the consequent too free use of analogy. It is by no means certain that the same mechanism holds for all cases, although the constancy of directive influence suggests this. But if the fundamental character of directive influence, as discussed above, be accepted, a uniformity of mechanism is no longer required.

Holleman assumes the intermediate formation of an addition product. Using the Kekulé formula for the benzene ring,



²⁶ Francis, *ibid.* 48, 655 (1926).

the substituent, X, is at one end of a conjugate double bond, 1-2-3-4 and either accelerates or retards the rate of addition to the conjugate system, according to Thiele's theory, and also to the simple bond, 1-2, but has little influence upon addition to the bond, 5-6. If the effect is acceleration, *ortho* and *para* substitution is favored; if it is retardation, *meta* substitution results. Van der Linden³⁷ has offered the objections that bromination of chlorobenzene ought to be more rapid than that of benzene itself; and that it should, on decomposition of the addition product, give some *p*-dibromobenzene as well as *p*-bromochlorobenzene. Both suppositions are contrary to fact. Moreover, Holleman has admitted³⁸ that no explanation is afforded as to why some groups accelerate and some retard the addition at the conjugate system, "the formation of intermediate addition products being even now hypothetical. Also the transition of these addition products into substitution products must be assumed as being performed with infinite velocity."

Recently Barnett³⁹ and his collaborators have defended Holleman's theory with evidence by analogy in the anthracene series. They maintained that Holleman's intermediate quinoid formula,



involves a spatial configuration which permits the elimination of only one pair of constituents (those which are *cis*) and so gives no possibility of the formation, for example, of *p*-dibromobenzene from chlorobenzene and bromine. These conclusions have been attacked by Prins⁴⁰ on the basis that the analogy fails because the central ring of the anthracene molecule has lost its aromatic character in the addition products which had been isolated; and that if benzene should lost its aromatic charac-

³⁷ Van der Linden, Verslagen Acad. Wetenschappen Amsterdam. Nov., 1911, p. 739.

³⁸ Ref. 2, p. 195.

³⁹ Barnett and Cook, *Rec. trav. chim.* **43**, 262 (1924); Barnett and Matthews, *ibid.* 530.

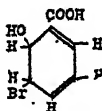
⁴⁰ Prins, *ibid.* 685.

ter in the same way, it would either polymerize, as do analogous terpenes, or add bromine to the double bonds. Furthermore, in the formation of the quinoid complex, there seems to be no reason why addition should be exclusively *trans*. This may be related to the theory of Terry and Eichelberger⁴¹ in regard to addition to double bonds. In their reply⁴² Barnett and Cook employed the Dewar formula for benzene, recently revived by Ingold and his collaborators.⁴³ They pointed out that compounds of the type,



are unknown when H and X have any considerable affinity for each other. But they admitted that their mechanism is not universal, and that in some cases there may be indirect substitution by way of a side chain.

The enhanced activity of halogens in aqueous solution has frequently been ascribed to the presence of hypohalous acids, which readily lend themselves to Holleman's mechanism by adding, supposedly, halogen and hydroxyl, and then eliminating the latter and a hydrogen atom in the form of water. If this mechanism were followed in the case of bromination of benzoic acid, for example, using Barnett's oriented addition, so as to place the hydroxyl group *cis* with respect to the hydrogen atom in the 5 position, the formula,



shows that the bromine atom would also be *cis* with respect to the hydrogen atom in the 6 position, and there certainly should be some elimination of HBr, leaving salicylic acid. In the medium employed, bromine water, this compound would be

⁴¹ Terry and Eichelberger, *J. Am. Chem. Soc.* **47**, 1067 (1925).

⁴² Barnett and Cook, *Rec. trav. chim.* **43**, 897 (1924).

⁴³ Ingold and others, *J. Chem. Soc.* **121**, 1153 (1922); **123**, 2066 (1923).

converted instantly to insoluble dibromosalicylic acid or tribromophenol, and could not fail of detection, even if present only in traces.

But even if, as Holleman maintains,⁴⁴ the failure of possible side reactions is no disproof of a mechanism, there is considerable evidence⁴⁵ that aqueous bromination is not due to hypobromous acid. A solution of the latter, if it is free from bromine, is much less reactive than bromine water, and shows mainly oxidizing rather than brominating properties. The suggestion was made that the high reactivity of bromine water is due to nascent bromine, that is, atomic bromine, either uncharged or charged positively; and this was supported by some results involving addition of halogens to double bonds. Br^+ (resulting from the partial ionization of Br_2 in aqueous solution) can be conceived as directly displacing a hydrogen atom of a benzene ring, and giving it its charge. The hydrogen ion thus formed would give hydrobromic acid with the Br^- ion, which had previously accompanied the Br^+ ion. This is a simpler but perhaps no more plausible mechanism for substitution. It does not account for nitration, unless indeed an NO_2^+ ion can be postulated.

There is thus involved the old controversy as to whether, with Michael⁴⁶ we believe that all reactions consist in the formation of complexes, followed by dissociation; or whether, according to Nef⁴⁷ dissociation into free radicals, is prerequisite. Possibly neither theory is universal.

The best known rule for directive influence, that of Crum Brown and Gibson, was stated⁴⁸ in entirely empirical form, namely, that a substituent, X, controls the *meta* or the *ortho-para* positions according to whether or not the compound, HX , can be oxidized directly to HOX respectively. More recently

⁴⁴ Ref. 2, p. 194.

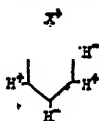
⁴⁵ Francis, *J. Am. Chem. Soc.* 47, 2340 (1925).

⁴⁶ Johnson and Hahn's Henrich's "Theories of Organic Chemistry." John Wiley and Sons, New York, 1922. Chap. XXI.

⁴⁷ Nef, *J. Am. Chem. Soc.* 26, 1549 (1904).

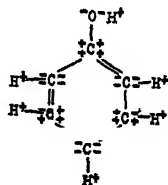
⁴⁸ Brown and Gibson, *J. Chem. Soc.* 61, 367 (1892).

Fry⁴⁹ explained it as follows: X in HX is assumed to be negative, while in HOX it is positive because of the negative character of the hydroxyl group. If the oxidation proceeds readily, it may be assumed that the tendency of X to be positive is greater than that to be negative. X will tend, therefore, to be positive in the benzene ring also, forming $C_6H_5-X^+$ rather than the "electromer," $C_6H_5^+X^-$. Assuming that the hydrogen atoms and substituents in the benzene ring alternate in sign,



the positive hydrogens will be, in this case, those *meta* to the substituent, X. These only are the ones which can be substituted, since hydrogen is removed as an element of an inorganic compound, such as HOH or HBr, in which it is always positive. If HX is not readily oxidizable, the tendency of X is to be negative, and the reverse reasoning applies. The positive or replaceable hydrogen atoms are now those *ortho* or *para* to X. In intermediate cases, there may be mixtures of the above electromers, so that mixtures of products result.

This explanation was criticized by Stieglitz⁵⁰ because it involves negative hydrogen atoms which should be powerful reducing agents, especially in the presence of a substituting agent such as chlorine. He explained the Brown and Gibson rule in terms of polarized bonds, showing phenol thus,



A reagent, such as HOCl, is supposed to add to the strongly polarized double bond, 1-2, or the conjugate system, 1-4, the

⁴⁹ Fry, *J. Am. Chem. Soc.* 36, 248 (1914).

⁵⁰ Stieglitz, *ibid.* 44, 729 (1922).

negative $-OH$ going to the highly positive carbon atom, 1, and then regenerating the double bond by combining with the H^+ atom at 2 or 4. This is evidently an electronic picture of Holleman's mechanism, mentioned above, and has the same objections.

Stieglitz may have misinterpreted Fry's formulas, in that a negative hydrogen atom held by a non-ionizable bond may have very different properties from those of a hypothetical negative hydrogen ion. Certain it is that Fry's theories account beautifully for many displacement reactions in aromatic chemistry, for example, the lability of halogen *ortho* or *para* to a nitro group. The latter, according to Fry, is positive, making the halogen more negative, in which form it is more readily removed as an ion by hydrolytic agents. Holleman⁵¹ made an exhaustive criticism of Fry's views, the most important points being the following:⁵² (a) *m*-Dichlorobenzene is hydrolyzed more readily than its isomers to chlorophenol, whereas, according to Fry, the two chlorine atoms would have the same sign in the *meta* isomer, and opposite signs in the *ortho* and *para* isomers, so that the reverse should be expected. (b) Since Fry accounts for the hydrolysis of benzene sulfonic acid in acid solution to give benzene and in alkaline solution to give phenol, by postulating that the $-SO_3H$ group is positive and negative respectively in the two media, and since the two acid groups of benzene *p*-disulfonic acid presumably have opposite charges, the compound should be hydrolyzed to phenol in either medium, while actually it behaves like the mono acid, giving benzene or quinol.

In his reply⁵³ Fry postulated that in both the cases cited the two groups have like signs, and that in (b) there is an unsymmetrical distribution of charges. In (a) the negative tendency of chlorine is reinforced by the coöperation of the two substituents, and the greater ease of hydrolysis may be due to this.

Reference has been made to the theory of indirect substitution by way of a side chain. Holleman and Euwes⁵⁴ showed the

⁵¹ Holleman, *ibid.* 36, 2495 (1914).

⁵² Van der Vliet also emphasized these points, *Rec. trav. chim.* 43, 606 (1924).

⁵³ Fry, *J. Am. Chem. Soc.* 37, 883 (1915).

⁵⁴ Holleman and Euwes, *Rec. trav. chim.* 27, 443 (1908).

improbability of this in the case of toluene since benzyl bromide does not rearrange to give *o*- or *p*-bromotoluene under conditions favorable to their formation. On the other hand, Holleman himself has found⁵⁶ that *o*-nitrobenzotrichloride upon hydrolysis, gives not *o*-nitrobenzoic acid, but *o*-chlorobenzoic acid, the nitro group being displaced by chlorine.

In the sulfonation of aniline Bamberger⁵⁶ has shown that aniline sulfate, phenyl sulfamic acid, and *o*-aminobenzenesulfonic acid are probably intermediate to the production of sulfanilic acid. Phenol is likewise supposed to be substituted in the *ortho* position by $-\text{COOH}$ ⁵⁷ or $-\text{SO}_3\text{H}$ ⁵⁸ only after the intermediate formation of the esters, $\text{C}_6\text{H}_5\text{OCOOH}$ and $\text{C}_6\text{H}_5\text{OSO}_3\text{H}$, respectively. Skraup and Poller⁵⁹ investigated the conversion of acetyl *m*-cresol to 2-methyl 6-hydroxyacetophenone, and considered that the migration of the acetyl group was due to resubstitution and not to indirect substitution, because in presence of *m*-chlorobenzoyl chloride, acetyl chloride was split out and a derivative of benzophenone resulted. They seem to have overlooked the possibility that the *m*-chlorobenzoyl group was first substituted for the acetyl group in $-\text{OH}$, and that it then rearranged, perhaps more easily than the acetyl group.

Chattaway and Orton⁶⁰ have shown that *N*-chloro-acetanilide, $\text{C}_6\text{H}_5\text{NCICOOCH}_3$, rearranges to give *p*-chloro-acetanilide, and that three chlorine atoms can thus be introduced into the 2, 4, and 6 positions by way of the $-\text{NH}$ group of acetanilide. They did not prove, as has been asserted,⁶¹ that halogenation takes place in the same manner in the case of free bases, although this is a natural inference.

More definite evidence for this mechanism, involving the

⁵⁶ Holleman, Vermeulen, and de Mooy, *ibid.* 33, 1 (1914).

⁵⁶ Bamberger, *Ber.* 23, 461 (1895); 30, 654, 1281, 2279 (1897).

⁵⁷ Schmidt, *J. prakt. Chem.* 31, 407 (1885).

⁵⁸ Baumann, *Ber.* 9, 55, 1715 (1876); 11, 1907 (1878).

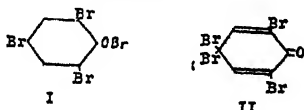
⁵⁹ Skraup and Poller, *Ber.* 57B, 2033 (1924).

⁶⁰ Chattaway and Orton, *Ber.* 32, 3573, 3635 (1899); *J. Chem. Soc.* 75, 1046 (1899); 77, 134, 789, 797 (1900).

⁶¹ Bernsthen—Sudborough's "Organic Chemistry." D. van Nostrand, New York. (1922). p. 395.

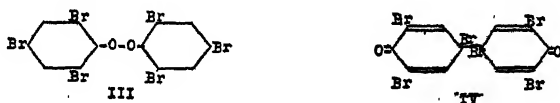
intermediate formation of $-\text{NHBr}$, $-\text{NBr}_2$, and $-\text{OBr}$ compounds in the bromination of amines and phenols is presented in my papers.^{19, 23, 34} It is shown, for example, that in aniline and in thirteen *m*-amino compounds and also in four derivatives of resorcinol, two of the three bromine substitutions are simultaneous, thus corresponding with the number of hydrogen atoms in the side chains. In no case is this number exceeded by the number of simultaneous substitutions. The mechanism is supported also by the peculiar dependence of the manner of bromination upon this number of hydrogen atoms.

Of important bearing upon this theory is the structure of tribromophenol bromide, to which Benedict⁶² assigned formula I, but which Thiele and Eichwede⁶³ claimed to be II, because



on long heating with lead acetate in acetic acid, it gave 2,6-dibromoquinone. This would seem to give opportunity for drastic rearrangements, however, while the rapid reaction with potassium iodide in cold aqueous solution, regenerating tribromophenol, decidedly favors Benedict's formula. Thiele failed to form from the compound a semicarbazone, the semicarbazide being oxidized and completely destroyed.

Kastle and his co-workers⁶⁴ made a careful study of the structure and decided in favor of Thiele's formula. They overlooked some of their own evidence, however. On heating to 125° , tribromophenol bromide loses one atomic equivalent of bromine forming $\text{C}_{12}\text{H}_4\text{O}_2\text{Br}_6$, which Benedict considered to be III, and Kastle IV.



⁶² Benedict, *Ann.* 199, 127 (1879).

⁶³ Thiele and Eichwede, *Ber.* 33, 673 (1900).

⁶⁴ Kastle, *Am. Chem. J.* 27, 31 (1902).

Kastle's co-workers considered the following facts to be proofs of the incorrectness of Benedict's formula: (a) the failure of tribromophenol bromide to form a benzene sulfonic ester with sodium benzene sulfinate; (b) its failure to form an ethyl ether with zinc ethyl; (c) its rearrangement in concentrated sulfuric acid to tetrabromophenol. (a) The observed reaction in the first case giving $C_{12}H_4O_2Br_6$ and $C_6H_5SO_2Br$ is no better explained by Thiele's formula. The powerful oxidizing action of tribromophenol bromide and the reducing tendency of sodium benzene sulfinate make it comprehensible with either formula. (b) In the second case the formation of ethyl bromide and zinc tribromophenolate is just what should be expected, and is more consistent with Benedict's formula than with Thiele's. (c) Kastle's mechanism for the rearrangement to tetrabromophenol is complicated, and subject to the objection that by it the sulfonic group might be expected to be left in the ring to some extent. Consideration of the well known fact that nitration of aniline in concentrated sulfuric acid gives largely the *meta* derivative, makes it reasonable to suppose that in the same medium the bromine attached to oxygen isomerizes into a *meta* position, especially when all ortho and para positions are filled, possibly because of the formation of an oxonium salt. It is not even necessary to assume Benedict's bimolecular mutual bromination, to which Kastle objects.

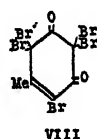
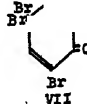
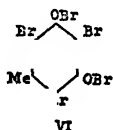
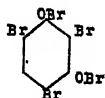
The formation of 2,6-dibromoquinone by boiling tribromophenol bromide with silver nitrate or lead acetate is merely analogous to the formation of quinone itself by oxidation of aniline or its *para* derivatives, since tribromophenol bromide is itself an oxidizing agent. Kastle's failure to cause the same reaction by means of silver oxide or calcium carbonate, nullifies this evidence for Thiele's formula. Kastle's other observations are explained equally well by either formula. A serious objection to that of Thiele is the failure of additional bromine to add to the double bonds of the quinoid ring, as it does to those of quinone itself.

The structure seems now to be settled satisfactorily as the "bromoxyl" formula by Lauer,⁶⁵ who showed that chlorination

⁶⁵ Lauer, *J. Am. Chem. Soc.* **48**, 442 (1926).

of tribromophenol gives a different product from that obtained by bromination of 2,6-dibromo-4-chlorophenol, in contrast to the requirement of Thiele's formula.

Let us consider now pentabromoresorcinol and pentabromo-orchinol, to which Benedict assigned formulas V and VI. No evidence has been advanced against these structures except the analogy with tribromophenol bromide. The compounds are more stable than the latter, but are readily reduced to tribromoresorcinol and tribromo-orchinol respectively, two of the bromine atoms being much more reactive than the other three. This lability is not shown as well by the more commonly used formulas, VII



and VIII. These structures have also the serious objection that they show one double bond in the ring, although there is no additional consumption of bromine even on long standing. On titration of resorcinol or orchinol with bromine in aqueous solution, a fairly good end point for *pentabromination* may be obtained, using as an indicator the odor of bromine or the color change of a moistened KI-starch paper suspended in the neck of the flask. The liquid reacts with potassium iodide after three molecular equivalents have reacted. Similarly, phloroglucinol reacts with six or three equivalents under corresponding conditions, but the excess over six equivalents is less permanent, since there is gradual oxidation with rupture of the ring.⁶⁶ The correspondence of the capacity for labile bromine with the number of hydroxyl groups, in phenol, resorcinol, orchinol, and phloroglucinol is striking support for Benedict's views.

GENERAL INFLUENCE OF SUBSTITUENTS

Studies in this field have been complicated greatly by the fact, not yet fully recognized, that some effect other than direc-

⁶⁶ Benedict, *Ann.* 189, 165 (1877); *Monats.* 6, 702 (1885); Zincke and Kegel, *Ber.* 23, 1729 (1890).

tive influence, as defined above, is exerted by substituents in the benzene ring upon the rate of further substitution. Thus it is well known that nitrobenzene is nitrated less readily than benzene, while phenol is much more reactive, and toluene slightly more so, than benzene. Holleman³¹ has described this by stating that the rate of *ortho* and *para* substitution is much greater than that of *meta* substitution. But chlorobenzene also is less readily substituted than benzene, although halogens are *ortho-para* controlling. That is, halogens in this respect are in the class with the nitro group and other "acid" groups, and not with $-\text{OH}$, $-\text{NH}_2$, and $-\text{CH}_3$.

This classification is parallel with that of the effect of groups upon the acidity of phenols, and the degree of dissociation of acids, and is commonly called the "negative nature of atomic groups." These influences are shown also in the researches of Norris³² and his students upon the reactivity of atoms not connected directly with the benzene ring.

It seems, therefore, that two or more influences of the groups are superimposed. These influences may be transmitted around the ring in both directions either with undiminished or with gradually decreasing intensity.³⁷ The capacity of the benzene ring for transmitting two or more influences may be due to the sharing by adjacent carbon atoms of the ring of *three* electrons^{37,38} instead of two, so that the induced shifts may be irregular; that is, the three electrons may not all shift together. This is analogous to the simultaneous transmission of two messages over the same wire at different frequencies. This will be discussed later.

This other influence, which is a property of various substituents, seems to result in an acceleration or retardation of substitution which is independent of the position of the group. For this reason I have called it "General Influence," and made an attempt to dissociate it from directive influence. The influences of some groups in amino and phenolic compounds are estimated¹⁹ as shown in table 2.

³⁷ Compare Højendahl, *J. Chem. Soc.* 125, 1381 (1924).

³⁸ Kermack and Robinson, *ibid.* 121, 437 (1922).

In phenolic compounds, as in benzene itself the general effect is a retardation for acidic groups and an acceleration for methyl (and, of course, for $-\text{NH}_2$ and $-\text{OH}$ if the experimental method had permitted their calculation). In amino compounds the general effect is always an acceleration, and is in nearly reverse order to that upon phenolic compounds. The general influence was estimated in amino compounds by comparing the mean of the first velocity constants of bromination of the *ortho* and *meta* isomers of a system with that of aniline. The directive influence was found by dividing this same mean by that of the *ortho* isomer. The former calculation thus eliminates the effect of position of the group, and the latter evaluates it.

TABLE 2
Influences of substituents in the benzene ring
(Effect upon first velocity constant of bromination)

SUBSTITUENT	AMINO COMPOUNDS		PHENOLIC COMPOUNDS	
	General	Directive	General	Directive
NO_2	3.4	0.14	0.0012	0.8
COOC_2H_5	3.7	0.22	0.001	
CHO			0.005	0.73
COOH	4.4	0.19	0.016	0.84
SO_3H	2.2	0.24		
Br	3.8	0.78		
Cl	3.8	0.78	0.065	4.0
CH_3	1.45	0.97	3.2	1.5

Other examples of this general effect can be found in the literature. Thus Wibaut⁶⁸ showed that toluene is nitrated in preference to chlorobenzene when mixed, although the directive influence of chlorine is greater than that of methyl when substituted in the same benzene ring. Holleman recognizes general influence in his factor, "X."⁶⁹

Olivier has made a thorough study of the rates of hydrolysis of various substituted benzyl chlorides.⁷⁰ His results can be

⁶⁸ Ref. 2, p. 206.

⁷⁰ Olivier, *Rec. trav. chim.* 41, 646 (1922); 42, 516, 775 (1923).

summarized in table 3, which gives the relative velocity constants at 83° compared with that of benzyl chloride itself as unity.

The order is evidently $p > o > m$ for *ortho-para* directing groups and the reverse for *meta* directing groups, showing the importance of position, and the close relation to directive influence of which p/m is a good index. The striking observation is, however, the fact that the halogen derivatives, like the meta controlling groups, all have constants below that of benzyl chloride instead of above, like methyl. In other words they have a *general* retarding effect like the other "acid" groups, but a special or directive influence like that of methyl. The results are analogous to my own, mentioned above.

I believe that disregard of the distinction between these two influences has been the cause of much confusion and some controversy regarding directive influence.

TABLE 3
Relative velocity constants of hydrolysis of benzyl chlorides

SUBSTITUENT	PARA	ORTHO	META	p/m
CH ₃	10.6	4.84	1.39	7.6
Cl.....	0.62	0.355	0.237	2.61
Br.....	0.50	0.286	0.215	2.34
I.....	0.477	0.287	0.201	2.38
COOH.....	0.165		0.245	0.68
NO ₂	0.074	0.084	0.090	0.82

STRUCTURE OF BENZENE

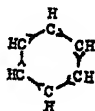
Obviously the chief difficulty about the structure of benzene is the distribution of the three extra bonds, or in modern terms, of the six spare electrons. I prefer to consider them as shared between carbon atoms, making three instead of two for each nuclear bond, and thus providing the mechanism for complex shifts, as already described. The actual positions of the three cannot be defined as yet. An attractive picture of the arrangement, because of its high symmetry, is that of Orelkin⁷¹ who placed the six spare electrons at the corners of an octahedron;

⁷¹ Orelkin, *J. Russ. Phys. Chem. Soc.* 54, 493 (1923). *C. A.* 18, 2501.

and at the same time at the mid-points of the lines connecting *meta* carbon atoms, the ring being "puckered," that is, the carbon atoms being in two different planes. In this arrangement two of these electrons form a perfect square with the two shared by two adjacent carbon atoms, simulating a double bond, except that the four electrons are not the exclusive property of the two carbon atoms involved. Morse⁷² placed the carbon atoms at the corners of an octahedron. This seems to be a mere speculation already disproved by chemical evidence.

Lely⁷³ has proposed a rather fantastical triangular formula, which must have some merit since it aroused, the criticism of no less than eight other chemists.⁷⁴ He attacked the Kekulé formula by claiming that the molecular refraction of benzene proves the absence of double bonds. This was denied vigorously by van Laar,⁷⁵ who showed that three double bonds are indicated by the value of the constant "a" in van der Waal's equation of state. Schoorl⁷⁶ stated that the molecular refraction of benzene corresponds with theory for three double bonds, but not if their conjugation is considered.

Dominikiewicz⁷⁷ has revived Stark's theory,⁷⁸ which involves the idea that two valences of one carbon atom satisfy one of the next.



This ought to be discredited because it implies the existence of isomeric *ortho* derivatives if the two substituents are different.

Huggins⁷⁹ and Beckenkamp⁸⁰ drew an analogy between the

⁷² Morse, *Phys. Rev.* 19, 243 (1922).

⁷³ Lely, *Chem. Weekblad.* 19, 593 (1922); 20, 90 (1923); 21, 114 (1924); 22, 371 (1925).

⁷⁴ *Ibid.* 20, 27, 28, 29, 143, 144, 145, 279, 297 (1923); 21, 174 (1924).

⁷⁵ van Laar, *ibid.* 22, 286 (1925).

⁷⁶ Schoorl, *ibid.* 22, 343 (1925).

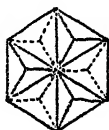
⁷⁷ Dominikiewicz, *Roczniki Chemje* 3, 326 (1923); *C. A.* 19, 2190.

⁷⁸ Ref. 46, pp. 209-225.

⁷⁹ Huggins, *J. Am. Chem. Soc.* 44, 1607 (1922); 45, 264 (1923).

⁸⁰ Beckenkamp, *Z. anorg. allgem. Chem.* 137, 249 (1924).

structure of benzene and that of graphite, based upon crystallographic data. From the structure assigned to graphite at that time, from X-ray spectra, Huggins assumed benzene to have a puckered hexagon formula, corresponding to Körner's centroid model.



Huggins defended this structure against objections that have been raised to it, (a) that di- and trisubstituted derivatives with different groups should show optical isomerism; (b) that substituents in *meta* positions should form closed rings, such as anhydrides, more readily than *ortho* substituents; (c) that 1,5 rather than 1,8 rings should be formed with naphthalene derivatives. He argued as follows: (a) The degree of optical rotation may be so small that experiments in the attempt to find it may have been insufficiently accurate or exhaustive. (b) Repulsion between *meta* substituents distorts the structure so that they are not really very close. (c) The only criterion for the numbering, 5,6,7,8 in naphthalene has been the 1,8 closed rings, so that, very probably, compounds such as acenaphthene and naphthalic anhydride are really 1,5 and not 1,8 derivatives.

It ought to be possible to test this last point by carrying out Fittig and Erdmann's synthesis⁸¹ of α -naphthol from γ -isocrotonic acid, using instead a compound with some substituent in the phenyl group, and comparing the resulting compound with known derivatives of α -naphthol. Huggins says, "Recent evidence from crystal structure proves this benzene structure to be the correct one." It has now been shown⁸² that graphite has a plane structure instead of a puckered one, so that a similar one for benzene is probable.

In contrast to Huggin's central accumulation of electrons, Crocker⁸³ considered that they are repelled to the outside, which

⁸¹ Fittig and Erdmann, *Ber.* 16, 43 (1883).

⁸² Hassel and Mark, *Z. Physik.* 25, 317 (1924); Bernal *Proc. Roy. Soc.* 106A, 749 (1924).

⁸³ Crocker, *J. Am. Chem. Soc.* 44, 1618 (1922).

is logical considering their electrical properties. The carbon atoms are thus placed in a single plane.

Pauling⁸⁴ has proposed a formula for benzene which is an electronic picture of the Claus formula since it involves " μ -electrons" which are shared between *para* carbon atoms. It is open to the objection that it assumes each carbon atom to be combined with *three* others, although in no case of rupture of the benzene ring is this condition realized.

PRINS' THEORY

The most recent comprehensive theory of substitution in the benzene ring is that of H. J. Prins,¹⁴ although it has had a gradual development⁸⁵ and is still stated somewhat cautiously. It is practically the same as that of Flürscheim,⁵ but stated in modern terms and freed from its principal objections. The paper should be widely read; it is in the English language.

The theory is stated in two ways which are equivalent; but the first involves "atom energy" and "bond energy," which are somewhat vague terms, and may not be generally acceptable, so that I prefer the other as being more readily explicable.

He considers that under the influence of a strongly electrophilic substituent (one with a pronounced affinity for additional electrons) such as chlorine, a carbon atom has less attraction for the electrons which it shares with the *ortho* carbon atoms. These electrons, therefore, are forced towards the latter atoms forming an octet, which includes also the other electrons around those atoms. The *meta* atoms coming thus under the influence of negative *ortho* atoms, repel their electrons toward the *para* atom forming an octet there. The general action of a negatively charged atom must be distinguished from the distribution of energy over the nucleus. Thus chlorine causes a general decrease of atom energy. This effect is strongest in the *ortho* atoms, and thus nearly neutralizes the distribution effect, so that the directive influence is almost exclusively *para*. An

⁸⁴ Pauling, *ibid.* 48, 1132 (1926).

⁸⁵ Prins, *Chem. Weekblad.* 14, 83 (1917); 15, 98, 571 (1918); *Rec. trav. chim.* 43, 685 (1924).

electrophilic atom indirectly bound to the nucleus as in $-\text{CCl}_3$, causes *meta* substitution; but this is not true for the tertiary butyl group, $-\text{CMe}_3$, because of the weakly electrophilic character of methyl. Prins does not make clear the disposition of the six extra electrons. There are no figures in his paper.

A THEORY OF SUBSTITUTION IN THE BENZENE RING

Substantially the same idea is contained in the following theory as elaborated by me. Indeed it can be considered as largely anticipated also by the theories of Flürscheim,⁵ Vorländer,³⁶ Fry,⁴⁰ Stieglitz,⁵⁰ and others^{67, 68, 87} if properly interpreted, but it seems to overcome some of the objections that have been offered to them. The following postulates are made:

1. Each nuclear bond of benzene consists of three shared electrons. The aromatic character is due to this. Each other single bond involves only two shared electrons. A shared electron presumably revolves around both atomic nuclei, but in the figures it is represented by a dot between the atoms. This may be considered the electric center of its orbit, but hereafter will be denoted merely by the term "electron."

2. Any substituent in benzene shifts the electrons of all the bonds. The three electrons of the nuclear bonds may not all shift together; but because of their pronounced tendency to pair, two of them may shift together, and the third be free to move in response to electrostatic influences, and even shift in a direction opposite to that of the pair. In most cases, for lack of data to decide the exact nature of the shifts, the three are represented together in the drawings. The direction and amount⁸⁸ of the shift depend upon the sign and degree of the polarity tendency of the substituent. The test for this is usually the relative stability of the hydrogen and hydroxyl compounds of the substituent (essentially the Brown and Gibson rule); but a safer criterion for groups, such as $-\text{CCl}_3$ or $-\text{CN}$, is to

³⁶ Vorländer, *Ber.* 52, 263, (1919).

³⁷ W. A. Noyes, *Ber.* 57, 1233 (1924).

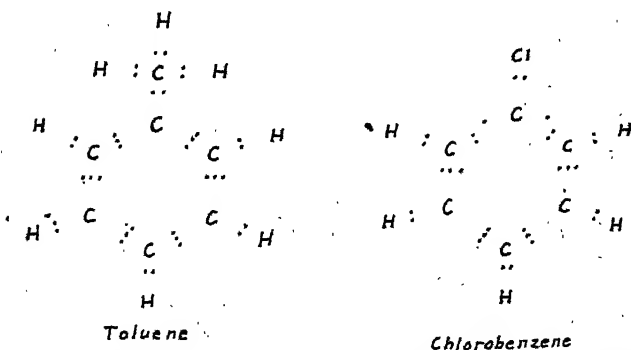
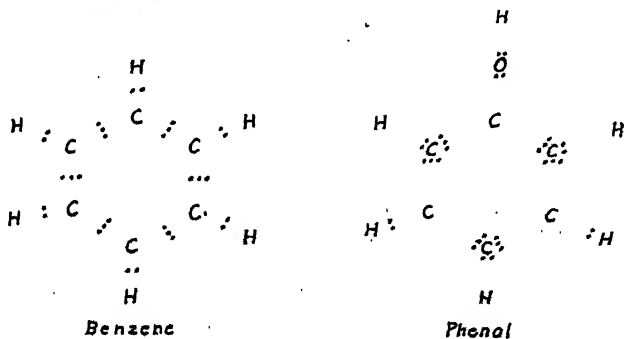
⁸⁸ The idea of gradual shift of shared electrons from those of a completely non-polar bond to those of an ionizable bond, and the method of representation of the bonds are due to G. N. Lewis, ref. 6, p. 83.

consider the polarity tendencies of the extreme atoms. This will be explained later.

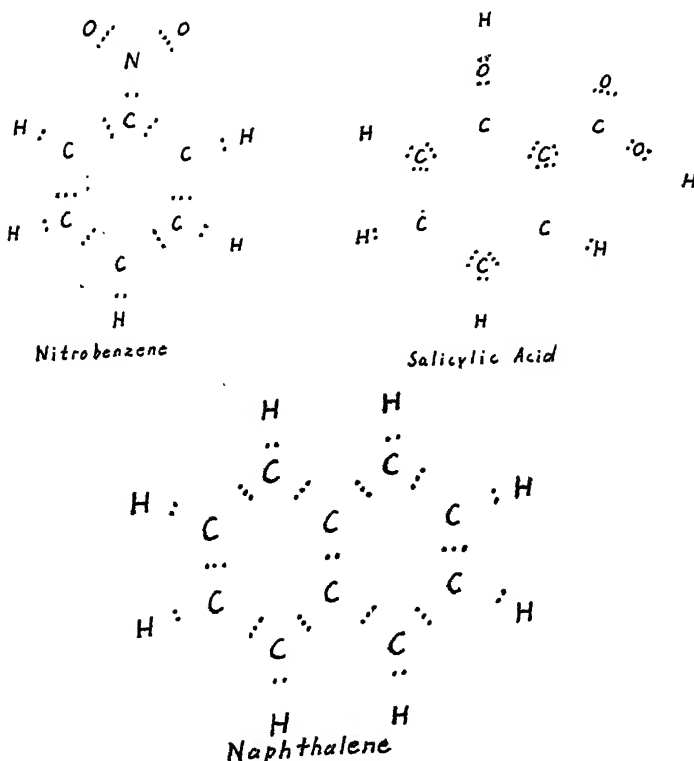
3. A carbon atom towards which electrons have been shifted tends to attract its other electrons forming a compact octet. A carbon atom away from which electrons have been shifted tends to repel its other electrons forming an open octet. This is based upon considerations of symmetry.⁸⁹

4. A bond is the more easily broken if the shift of its electrons is in such a direction as to accentuate the normal polarity of the atom or group to be displaced. Thus, hydrogen is easily displaced if electrons are shifted away from it, while a halogen atom is labile if electrons are shifted toward it.

The following figures illustrate the theory:



⁸⁹ This is contrary to the assumption of Lewis (ref. 6, p. 85) that all the valence electrons of a molecule are shifted in the same direction, but is suggested by him as a possibility (p. 150) in certain cases. His first assumption is inapplicable to substitution in the benzene ring. Cf. also Lapworth, *Mem. Manchester Phil. Soc.* 64 (iii) 1 (1920); *J. Chem. Soc.* 121, 416 (1922); *C. A.* 16, 2047-8.



Hydroxyl tends to be negative, and in order to acquire that polarity, attracts the electrons which it shares with carbon. The latter then repels its other six electrons, forming an open octet. The two *ortho* carbon atoms then have compact octets, the two *meta* carbon atoms open ones, and the *para* atom a compact one. The more positive hydrogen atoms are the *ortho* and *para* ones, and these are the ones substituted. In aqueous solution they have practically the reactivity of ions.³⁶ In toluene the arrangement of electrons is analogous with that of phenol, but the shifts are much less extensive because of the weaker polarity tendency of methyl. In chlorobenzene the shifts of the electron pairs are similar to those in toluene, but the third electrons in the 1-2 and 1-6 bonds are attracted directly by the chlorine atom, and so shift in the opposite direction to that of the

pair. This distortion of the octets of the *ortho* carbon atoms greatly decreases the lability of the *ortho* hydrogen atoms, so that there is a general decrease in reactivity, and substitution is preferably in the *para* position.

In nitrobenzene the nitro group tends, as in nitric acid, to be positive, and the shifts are opposite to those in toluene. The *meta* hydrogen atoms are more labile. In nitrochlorobenzenes, however, chlorine atoms in the *ortho* and *para* positions would be the ones displaced because they are more negative. Similarly the *meta* isomer of the dichlorobenzenes is most readily hydrolyzed because the two chlorine atoms reinforce and accentuate each other's negative character, whereas in the *ortho* and *para* isomers they oppose each other, and so are nearly neutral.

The carboxyl group of salicylic acid is easily displaced because it is forced by the hydroxyl group to be strongly positive, and thus more readily combines with an hydroxyl ion forming carbonic acid. Similar reasoning applies to the $-\text{COOH}$, $-\text{CHO}$, $-\text{CH}_2\text{OH}$, and SO_3H groups when *ortho* or *para* to an amino or hydroxyl group. $-\text{CH}_2\text{OH}$ is somewhat anomalous, since it is an *ortho-para* directing group, and so in benzyl alcohol must be slightly negative. It may be that it is labile in salicyl alcohol only in a strongly oxidizing medium such as bromine water. The by-product in this case is formaldehyde, formed presumably from the unstable bromomethanol. Thus, when the weakly negative tendency of the group has been overcome by hydroxyl, and become positive, it is attracted away from the nucleus by negative bromine, being replaced by positive bromine.

Analogous reasoning accounts for the fact that benzene sulfonic acid, which in acid solution can be hydrolyzed to benzene and sulfuric acid because of the normal positive character of $-\text{SO}_3\text{H}$, is hydrolyzed by alkali to phenol and sodium sulfite, since the strongly positive sodium ion overcomes that slightly positive character, and combines with the group. The same idea applies to benzene *p*-disulfonic acid, thus meeting the criticism of Fry's theory by Holleman and van der Vliet. There is no probability that one $-\text{SO}_3\text{H}$ group is positive and the other

negative. A perfectly symmetrical structure for naphthalene is given, without the elimination of the 9-10 bond,⁶⁴ for which there is no justification; and the slight preference for substitution in the α -position is indicated. Other cases of orientation in naphthalene are analogous to those in benzene.

In considering Olivier's results on the hydrolysis of benzyl chlorides, we have two pictures of the $-\text{CH}_2\text{Cl}$ group,



If the group is in such a position with respect to other substituents that hydrogen would be reactive (positive), the group also would be positive as in (a). The carbon atom has an open octet and the negative character of chlorine is accentuated, so that it is labile. If the group is in a different position, *meta* to an *ortho-para* directing group, for example, the carbon atom has a compact octet, (b), and the chlorine atom is more positive and less readily hydrolyzed. This corresponds precisely with Olivier's observations.⁷⁰

Reference has been made to the $-\text{CCl}_3$ and $-\text{CN}$ groups which are obvious exceptions to the Brown and Gibson rule, since they are *meta* directing, although their hydrogen compounds cannot be oxidized to their hydroxyl compounds. If we write their electronic formulas however,



we see that the combined action of the chlorine atoms in the former case, and the negative tendency of trivalent nitrogen in the latter, impose an open octet upon the carbon atom, which is thus positive and *meta* directing. The tertiary butyl group, $-\text{CMe}_3$, would be expected to be analogous to $-\text{CCl}_3$; yet it is *ortho-para* directing. Prins' explanation of this case has already been mentioned, namely, that the weakly electrophilic character of methyl is not transmitted through the carbon atom. A some-

what similar reasoning applies to other substituents involving aliphatic chains (for example, the cinnamic acid group), all of which are *ortho-para* directing. It would seem that in some cases a carbon chain has considerable damping effect upon the transmission of induced alternating polarity.

The theory explains all the phenomena cited by Fry in the same way that he does, but avoids some objections to his theory. One is that he does not make it clear why one positive hydrogen should be attacked more rapidly than another; why, for example, phenol should be so much more reactive than chlorobenzene. This is readily explained by assuming that by gradual shift of the electrons many gradations of positivity or negativity are realized. There are no strongly negative hydrogen atoms, but only more or less positive, or slightly negative ones.

PHENOL TESTS

I. A CLASSIFICATION OF THE TESTS AND A REVIEW OF THE LITERATURE

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INTRODUCTION

Chemists and biologists have found extensive use for tests of phenolic compounds. Compounds of this class are not only widely employed in industry but are distributed in liberal variety throughout the animal and vegetable kingdoms. Many are found by the biologists to occur naturally as products of metabolism; several are used as medicinals or antiseptics.

Their varied importance led to many methods of detection; and, since quantities available were often small, there has been continual effort to develop delicate color tests and wherever possible to make the tests specific. More than 40 of the tests vary in the character of the reagents, and these have been modified, improved and complicated in various ways so that now the tests described in the literature number more than 100.

It is of interest to note that while these tests are designed to detect phenols, very few of the reactions employed involve the hydroxyl group directly. They are brought about, mostly, through the influence of the phenolic group upon the remainder of the molecule, resulting, in a large number of instances, in ring substitution para to the hydroxyl group.

In some cases the hydroxyl group is modified in the course of the reaction, as in the case of oxidation to quinone, the nitroso reaction to oxime, the production of urethans, and in alkylation or acetylation. In practically all of the other tests the hydroxyl group remains intact in the final product of the reaction, subject,

naturally in many cases, to the wandering of the labile hydrogen to produce tautomers. The oximes, produced by the nitroso reactions, are examples of the latter. (See second paper of this series.)

In cases where one or more of the hydroxyl groups remain in the final product, the acidic properties of this group determine the conditions under which color appears or changes of color take place.

It is to be noted that many of the tests enumerated for the phenolic group are produced with equal, or greater, facility by the amine group, in which case the reactions are usually much more complicated and quite apt to take place with the amine group directly.

The classification of many of the reactions involved in the phenol tests is simple; in some cases there is doubt regarding the exact character of the color formed, and in other cases no information is available upon which a logical classification can be based. I have endeavored to collect the important literature on the subject, and group the tests for purposes of simplicity and classification according to the chemistry of the reactions involved, wherever expedient.

A few of the tests have been satisfactorily applied on a quantitative basis for certain phenols; but the large majority is not susceptible of quantitative application, is not qualitatively specific for any phenol, and some of those that have been most employed by investigators are not specific for phenols as a class but are also given by many other substances not phenols. In employing such tests it is necessary that the investigator first be advised by some other means concerning the character of the material to be investigated. It is only then that many of the tests can be of any value and capable of intelligent application.

The very extensive literature treating of these tests is so widely scattered throughout chemical and other journals that much of it is not available to biologists. While the most of it is here presented no effort has been made to collect it all. However, it is believed that the references appended to this article together with those cited in the references will prove to comprise a fairly

complete list. Since an article on the indophenols is in preparation, which will include extensive references to these compounds, only the necessary references to this very interesting class of substances are here included.

In addition to the literature compilation the chemistry of the first group, the nitroso colors, has been elucidated by the work described in the second paper of this series. This group includes the many and complicated color tests employing dilute nitric acid, nitrous acid, alkyl nitrites and the various reagents of this class containing mercury salts, Millon's, Hoffmann's, Plugge's, Lintner's reagents and others.

In a later paper a new application of the indophenol test will be elucidated. It has been studied quantitatively and has been found to be very delicate for some phenols. This review is presented with the hope that it will be of assistance to chemists and biologists and also for the purpose of giving perspective to the experimental work now in progress and which will be presented in subsequent articles.

CLASSIFICATION OF TESTS

A. Dye reactions

A considerable number of the color reactions employed for the detection of phenols can be classified as producing dyes of one of the following six groups.

1. Nitroso colors
2. Nitro colors
3. Azo colors
4. Di- or triphenylmethane colors
5. Xanthene colors
6. Indophenol colors

These will be considered in the order named.

1. *Nitroso colors.* Nitrous acid reacts with many phenols with the production of compounds giving colored solutions of nitroso derivatives. In the presence of a condensing agent, such as sulfuric acid, the nitroso compound is condensed with the phenol giving the well known Liebermann, indophenol test.

It has been very difficult to differentiate the various tests where nitrous acid is employed and to classify them definitely. In the case of some indophenol tests both classes of colors are undoubtedly present, and in other cases condensation of the nitroso derivatives may take place in other directions.

In the case of certain tests, Nickel (1890), without experimental evidence, expressed the opinion that red dyes were formed from the nitrosophenols produced in the test, and in the case of salicylic acid Lintner (1900) holds the same view.

Tests employing dilute nitric acid are also described. It seems improbable under the conditions, that the nitric acid is present in sufficient concentration to cause nitration and it appears most probable that the color is due to nitrous acid present in the nitric acid or formed by the reducing action of organic compounds present.

It is interesting in this connection to note the action of nitrogen tetroxide and nitrogen pentoxide. Houston and Johnson (1925) found that the former acts as nitrous acid (nitrosyl nitrate) while Haines and Adkins (1925) found the latter to be an excellent nitrating agent.

The use of nitric acid mixed with sulfuric acid or hydrochloric acid is also described. In the case of sulfuric acid there is produced a nitrating mixture and this test is described under the nitro colors. In the case of hydrochloric acid very active chlorine is liberated and while there may be the effect of nitrous acid on the phenol this test is more fully treated under the halogen tests.

All of these reactions except those definitely belonging to the indophenol or nitro class are described under this heading. This classification is justified as a result of some of the experimental work described in the second paper of this series.

a. Colors formed by dilute solutions of nitrous acid, nitrites (salts of nitrous acid, alkyl nitrites, ethyl nitrite, amyl nitrite).

See previous discussion.

b. Colors formed by dilute solutions of nitric acid.

I have found that the use of the ordinary best reagent grade of nitric acid diluted, 3 drops in 10 cc. of water, will give a color

with a phenol solution, 2 parts in 1000, when an equal volume of the dilute acid is added. The color which appears on standing 10 or 15 minutes appears the same as that produced by dilute solutions of nitrous acid. (See second paper of this series.)

c. Colors formed by dilute solutions of nitric acid in presence of various salts (mercuric sulphate, etc.).

This test is similar to the preceding except for the catalytic action of the mercury salts.

d. Colors formed by nitric acid and nitrous acid in presence of salts of mercury and some other metals.

In this group is classed the very important and much used Millon's reagent (a nitric acid solution of mercury) made in various slightly modified ways, Hoffmann's reagent (a solution of mercuric oxide in nitric acid with the addition of a trace of nitrous acid), Plugge's reagent (a solution of mercurous nitrate with the addition of some free nitrous acid) and Lintner's reagent (a solution of mercuric nitrate, sodium nitrite, acidified with sulfuric acid).

See second paper of this series.

References

- | | |
|------------------------|-------------------------------|
| Millon (1849) | Perkin and Nierenstein (1905) |
| Hoffmann (1853) | Bach (1911) |
| Meyer (1864) | Elvove (1917) |
| Griessmayer (1871) | Weiss (1920) |
| Plugge (1872) | Chapin (1920) |
| Almén (1877) | Sen-Cupta (1921) |
| Allen (1878) | Salus (1922) |
| Hirschsohn (1881) | Huerre (1922) |
| Eykman (1882) | Ware (1925) |
| Fresenius (1883, 1889) | Houston and Johnson (1925) |
| Nickel (1890) | Haines and Adkins (1925) |
| Lintner (1900) | Ekkert (1925) |

2. Nitro colors. a. Nitration test. Many phenols can be nitrated to colored nitro derivatives. There is usually employed for this purpose a nitrating mixture of sulfuric and nitric acids. This reaction is one of the standard tests that has been used for a long time. In the case of phenol, picric acid results and by color comparisons of the acid or of its more highly colored salts with standards the test is made roughly quantitative.

References

Mulliken (1914, pg. 108)
 Sen-Gupta (1921)

Huerre (1922)

3. *Azo colors.* The Peter Griess diazo reaction and the coupling of the diazo derivative with phenols to form dyes has been found to be susceptible to development for its great delicacy as tests for phenols and for that reason has been extensively employed.

a. Diazotized sulphanilic acid coupled with various phenols. Ehrlich made use of diazotized sulphanilic acid and other diazotized amines in testing biological material but Hanke and Koessler first attempted to develop the test on a quantitative basis.

b. Diazotized paranitraniline coupled with various phenols. This test, as applied in a qualitative way, has been very popular but it has remained for Palkin and Wales to add a very valuable contribution in recording the spectroscopic investigations of 45 phenols most encountered in pharmaceutical preparations, which should render certain the identification of many of these compounds.

References

Ehrlich (1882, 1883, 1884)
 Penzoldt and Fischer (1883)
 Nickel (1890)
 Limpricht (1891)
 Amann (1896)
 Riegler (1899)
 Pauly (1904, 1905)
 Formanek (1905, 1908, 1913)
 Malacarne (1907)
 United States Pharmacopoeia, 9 (1916)
 Formanek and Knop (1917)

Chapin (1920)
 Fox and Guage (1920, 1922)
 Hanke and Koessler (1920, 1922)
 Bell (1921)
 Moir (1922)
 Ellms, Marshall and Phillips (1922)
 Theis and Benedict (1924)
 Palkin and Wales (1924, 1925)
 Wales and Palkin (1926)
 Pyman (1926)

4. *Di- or triphenylmethane colors.* Various methods and reagents have been employed to perform this dye test. They produce brilliantly colored solutions of compounds which, in most cases, I believe should be classified in this group. The characteristics of the colors produced in many of the tests never have been sufficiently investigated, but from the mode of per-

forming the tests, dyes analogous to the triphenylmethanes will be produced.

In general the test consists in the condensation of an aldehyde with a phenol. A large number of aldehydes has been employed, such as formaldehyde, acetaldehyde, other alkylaldehydes, methyl glyococoll, vanillin, furfural, benzaldehyde, p-hydroxybenzaldehyde and others.

Other compounds producing reactions comparable to the aldehydes, such as phosgene, iodoform, bromoform, chloroform, chloral, carbon tetrachloride, oxalic acid, dihydroxyacetone, etc., have been employed.

As condensing agents sulfuric acid, hydrochloric acid, alkalis and other substances have been used.

The most generally employed reagents are formaldehyde and sulfuric acid.

- a. *Marquis' reagent: Formaldehyde and sulfuric acid.*
- b. *Ehrlich's reagent: Benzaldehyde and hydrochloric acid.*
- c. *Guaraschi's test: Chloroform and alkali.*

References

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|----------------------------|--|
| Kolbe (1861) | Lambert (1892) |
| Baeyer (1872) | Marquis (1896) |
| Guaraschi (1872) | Hehner (1896) |
| Miehler (1876) | Barbet and Jandrier (1896) |
| Doebner (1878, 1879, 1880) | Endemann (1897) |
| Nencki and Schmid (1881) | Melzer (1898) |
| Saraaw (1882) | Deniges (1898, 1909, 1911) |
| Nencki and Sieber (1882) | Hartwich and Winckel (1904) |
| Lustgarten (1882) | Rhein (1907) |
| Ihl (1885, 1889) | Silbermann and Ozorovitz (1908) |
| Udránszky (1888) | Pouget (1909) |
| Schwarz (1888) | Sanchez (1911) |
| Crismer (1888) | United States Pharmacopoeia (1916,
pg. 619) |
| Raupenstrauch (1888) | Sieburg (1916) |
| Geigy (1889) | Loele (1920) |
| Nickel (1890) | Salus (1922) |
| Desesquelle (1890) | Gomberg and Snow (1925) |
| Caro (1892) | |

5. *Xanthene colors.* a. *Phthalic anhydride fusion.* This test is essentially a fusion of phthalic anhydride with the phenol in

presence of a condensing agent. The phenolphthaleins long have been known but the colors of various derivatives were not systematically investigated until the work of Gsell, and Formanek and Knop. The former measured the absorption spectra in the visible region of 20 phenolphthaleins and the latter investigators presented a critical review of the work and also prepared some phenol anthraquinone derivatives.

Many phenols can undoubtedly be thus identified but this means of investigation is limited to cases where a considerable quantity, (e.g., 0.1 g.) of the phenol is available to carry out the fusion.

References

- | | |
|--------------------------|----------------------------|
| Baeyer (1876) | Formanek and Knop (1917) |
| Mulliken (1914, pg. 107) | Orndorff and Murray (1917) |
| Gsell (1916) | |

6. *Indophenol colors.* The name "indophenols" was given by Köchlin and Witt to a class of dyes developed by them about 1880; but long before this, about 40 years, colors were produced in various ways which we now recognize as due to indophenols.

These colors can be produced by various reagents applied in a variety of ways and the tests can be made very delicate. The acid solutions of the free indophenols are red while the solutions of the alkali and ammonium salts are intense blue.

It is interesting to note that Parry (1924) ascribes the color produced by Mandelin's reagent (1884), a vanadate in sulfuric acid, to the formation of a vanadium type of indophenol colors $O:C_6H_4:VH \cdot C_6H_4 \cdot OH$.

Previous to the work which will be described in a later paper of this series the tests have not been developed on anything but a qualitative basis. The new procedure depends upon indophenol formation which has been developed to a very delicate quantitative basis.

a. Liebermann test. Concentrated sulfuric acid containing nitrous acid. By this test the phenol is first partially converted to p-nitrosophenol which condenses through the agency of the sulfuric acid, with the phenol to form the indophenol.

Eijkman (1883) and Gutzkow (1889) employed amyl nitrite and sulfuric acid.

b. The simultaneous oxidation of an amine (p-aminophenol) and a phenol by a great variety of oxidizing agents will produce an indophenol. The oxidizing agent most commonly employed is hypochlorous acid.

c. Fluckiger test. This test depends upon the reaction of bromine upon the ammonium salt of a phenol. It produces an intense blue color and while it has not been investigated I believe it should be classed here and that the reaction is due to the bromine oxidation of the ammonium salt of the phenol rather than bromine substitution in the ring, although both may take place. It is well known that many oxidizing agents, including atmospheric air produce this reaction.

d. Quinonechloroimide tests. These tests employ various quinonechloroimides reacting with certain phenols to form indo-phenols and are fully treated in the experimental part of a later paper.

References

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|-------------------|---------------------------|
| Berthelot (1859) | Hager (1884, 1885) |
| Lex (1870) | Waller (1887) |
| Neubauer (1872) | Wurster (1887) |
| Salkowski (1872) | Gutzkow (1889) |
| Jacquemin (1873) | Neubauer and Vogel (1890) |
| Rice (1873) | Manseau (1901) |
| Fluckiger (1873) | Kühl (1905) |
| Liebermann (1874) | Raschig (1907) |
| Cotton (1874) | Rodillon (1921) |
| Pollacci (1874) | Frieber (1921) |
| Almen (1877) | Parry (1921, 1924) |
| Eijkman (1883) | |

B. Halogen reactions

Halogenation tests have been applied extensively qualitatively and on a quantitative basis for detecting and estimating phenols. In many cases the methods are of considerable value in determining the identity of phenols, when sufficient quantities are available, since precipitates may be formed which can be purified and identified.

a. Iodination. Iodinated compounds of various phenols long

have been known and the methods of preparation are numerous; among which may be mentioned the use of iodine dissolved in alkali, iodine in potassium iodide solutions, iodine and mercuric oxide, iodine in sodium phosphate, iodine monochloride, potassium iodide and hypochlorite solution, and arylsulphonhalogenamides.

Cofman (1919) has shown that the active substance in the iodination of phenols is hypiodous acid, HOI, and that any method for the preparation of hypiodous acid can be used for the preparation of iodophenols.

As a quantitative method it has proved in most cases unsatisfactory although for some phenols satisfactory methods have been worked out, Emery (1921). See also Volumetric Iodate Methods, Jamieson (1926).

b. Bromination. Brominated derivatives have been employed both for the quantitative estimation and qualitative detection of phenols. As reagents bromates, bromites and free bromine have been employed.

c. Chlorination. The contamination of city water supplies by phenols from by-product coke ovens, and chemical works of various kinds has been evident by the objectionable odor and taste resulting from the chlorination of these waters either by hydrochlorite or free-chlorine according to water works practices. Comparisons of some of the various tests for phenols by Ellms, et al., lead them to the conclusion that the odor and taste tests are the most delicate for the purposes of water investigation.

The so-called euchlorine test employs potassium chlorate and concentrated hydrochloric acid. A reaction produced by a mixture of nitric and hydrochloric acids is classified under this heading since the reagent will generate chlorine. However, the test may well be attributed to the action of nitrous acid. The compounds formed have not been investigated.

Arylsulphonhalogenamides, —chloramine T (p-toluenedisulphochloroamide), halazone (p-sulfodichloraminobenzoic acid), and compounds of the type $\text{Ar} \cdot \text{SO}_2\text{NXM}$ where X = halogen and M = sodium or potassium, have been employed in halogenation, giving colors with various phenols.

Vitali (1892) describes a test employing a reagent consisting of concentrated sulfuric acid into which are put a few crystals of potassium chlorate. On adding a few drops of the solution to be tested there is first developed a green color changing to blue, if phenol is present.

I have tried this test and find that it is given by two drops of an 0.008 M (1:1,300) phenol solution. The mechanism of the reaction has not been investigated but it is natural to attribute it to chlorine dioxide. It may not be correct to list this test under the head of chlorinations but it is placed here for want of a better classification. If the sulfuric acid is contaminated with small quantities of oxides of nitrogen the indophenol reaction must be considered as a possibility.

References

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| Landolt (1871) | Wilkie (1911) |
| Koppeschaar (1876) | Hensel (1912) |
| Almén (1877) | Schewket (1913) |
| Allen (1878) | Mulliken (1914, pg. 109) |
| Hirschsohn (1881) | Folin and Dennis (1915) |
| Waller (1881) | Guglielmelli (1917) |
| Jacobson (1886) | Cofman (1919) |
| Vitali (1892) | Berthelot and Michel (1919) |
| Kossler and Penny (1893) | Desvernes (1920) |
| Neuberg (1899) | Emery (1921) |
| Kahn (1907) | MacLean and Thomas (1921) |
| Dehn and Scott (1908) | Ellms and Lawrence (1922) |
| Bougault (1908) | Heckner (1922) |
| Skirrow (1908) | Ellms, Marshall and Phillips (1922) |
| Mascarelli (1909) | Roberts (1923) |
| Mooser (1909) | Blaque (1923) |
| Neuberg and Hildesheimer (1910) | Vortmann (1923) |
| Liechti and Mooser (1911) | Jamieson (1926) |
| Seidell (1911) | |

C. Salts of metals

Under this heading there are classified a large number of tests alphabetically arranged according to the metal employed in the reagent. Attempts to work out the chemistry of the reactions employed have been recorded in the literature in only a few cases.

A most natural arrangement of many of these reagents would

place them definitely in the oxidizing class, the most obvious examples of which are, ferric chloride, dichromate, permanganate, etc. While such reagents undoubtedly act as oxidizing agents, the color formation is not to be explained so simply. Colored complexes may be formed with the metallic compounds, as has been definitely shown in the case of iron and titanium, and various condensations may take place with the oxidized phenols forming complex quinone derivatives. A number of the compounds of the metals entering into the reagents produce highly colored derivatives on reduction. In general it may be stated that in almost every case the color of the test is due to the reducing action of the phenol upon the salts of the metal or metals present and that the color of the oxidized phenol is not the predominating color of the reaction. It is therefore evident that in very few cases can these tests be made specific for any phenols and in fact the majority of them is undoubtedly not specific for phenols as a class, since any reducing substance will, in most cases, produce colors in the solutions of the reagents.

Antimony. A sulfuric acid solution of an oxide was employed by Levy (1886, 1887) to test a number of phenols and alkaloids. A number of other metals was also employed in the same way. See arsenic, bismuth, columbium, molybdenum, tantalum, tin, titanium and vanadium.

Arsenic. Angelo (1922) employed disodium arsenate (Na_2HAsO_4) and diammonium phosphate, $(\text{NH}_4)_2\text{HPO}_4$, either one producing a color when a small crystal is put into a 2 per cent phenol solution and then a small quantity of hydrogen peroxide added. The salts probably act as catalysts for the oxidation. See hydrogen peroxide test.

Guglielmelli (1916, 1917, 1918) devised tests employing arsenotungstic acid and arsenotungstomolybdic acid and compares the delicacy with 20 other of the known reagents. See also Lévy (1886, 1887).

Bismuth. See Levy (1886, 1887).

Cerium. Cerium sulphate gives a color with various phenols and it is stated that phenol can be made a very delicate test for cerium, 1 to 2000. Orlow (1907).

Chromium. Potassium dichromate has been employed as a reagent.

Cobalt. Bellucci and Chincini (1919) report that α -nitroso- β -naphthol gives a blood red color with cobalt compounds, is a very sensitive reagent and can be made the basis of a quantitative, colorimetric method for cobalt. Under certain conditions precipitates are obtained not only with cobalt but with other metals. Nickel (1888); Guglielmelli (1917).

Columbium. See Lévy (1886, 1887).

Gold. Auric chloride gives color reactions with phenols. Guglielmelli (1917); Fresenine (1883); Hirschsohn (1881).

The iron reagents. a. Ferric chloride. The blue color produced by ferric chloride reacting in solutions of many phenols has been a popular test for a long time. However, it is not very delicate. Sarauw (1882). Rashig (1907); Kahn (1907); Almén (1877).

Weinland and Binder (1912) describe certain phenol-iron complexes.

References

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|--------------------|------------------------------------|
| Waller (1902) | Hantzsch and Desch (1902) |
| Sarauw (1882) | Rashig (1907) |
| Hagar (1884) | Gibbs (1908) |
| Nickel (1890) | United States Pharmacopoeia (1910) |
| Bonanni (1900) | Wienland and Binder (1912) |
| Desmoulière (1902) | Mulliken (1914) |

a. Ferric alum. A solution of ferric alum is often employed in place of ferric chloride. United States Department Agriculture Bureau of Chemistry Bulletin No. 107 (1907).

b. Ferricyanide and ferric chloride. Jendrassik's (1923) reagent, consisting of potassium ferricyanide and ferric chloride, was found by Bezssonoff (1924) to be sensitive reagent for o- and p-phenols due to the reducing action of the phenols forming Prussian blue.

c. Ferricyanide and ammonia. This reagent has been employed by Candussio (1900). Pummerer et al. (1922, 1925) isolated a colorless ketone by oxidizing o-cresol with potassium ferricyanide in sodium carbonate solution.

d. Ferrous sulfate in presence of tartrates has been employed by Mitchell (1923), Price (1924) in the quantitative estimation of certain phenols found in galls and tannins.

Manganese. Potassium permanganate in alkaline solution will oxidize many phenols. Mulliken (1914, pg. 79.)

Mercury. Denige's reagent (1898), an acid solution of mercuric sulfate, has been employed by Escaich (1920) to distinguish between α - and β -naphthol, since α -naphthol gives a brilliant color while β -naphthol forms a yellow precipitate.

Millon's reagent, mercury dissolved in nitric acid, and other reagents of the same type containing mercury, are treated under the heading of the nitroso dyes.

Molybdenum. Fröhde's reagent is a solution of molybdic acid in sulfuric acid. Davy (1878), Allen (1878), Guglielmelli (1917), Lévy (1886). For the tungsten-molybdenum phosphoric acid complexes see tungsten.

Nickel. Color compounds of the type $\text{Ni}(\text{CN})_2 \cdot \text{NH}_3 \cdot \text{C}_6\text{H}_5 \cdot \text{OH} \cdot \text{H}_2\text{O}$ are described by Hofmann and Höchtlen (1903).

Phosphorous. Angelo (1922) employed diammonium phosphate and hydrogen peroxide in testing phenols. The former probably acts as a catalyst in the oxidation. See hydrogen peroxide test.

Platinum. Platinic chloride in some cases reacts somewhat similarly to gold. Fresenius (1883); Hirschsohn (1881).

Selenium. Levine (1920) states that selenious acid is a sensitive reagent for phenols and is of wide applicability.

Silver. Tollen's reagent, an ammoniacal solution of silver nitrate, is reduced by some phenols, especially many polyphenols. Mulliken (1914, fig. 22); Escaich (1920).

Sodium. Sodium peroxide has been employed as a test by Alvarez (1905).

Tantalum. See Lévy (1886, 1887).

Tin. See Lévy (1886, 1887).

Titanium. Hauser and Levite (1912, 1915) state that titanium salts form stable, colored complexes with phenols when two hydroxyl groups are present in the ortho position. An arseno-titanium reagent has been recommended by Guglielmelli (1917).

who also employed a sulphotitanium test. Also see Lévy (1886, 1887); Déniges (1916).

Tungsten. Tungstic acid has been employed in tests for phenols; however, the test most employed has made use of a reagent composed of tungsten and molybdenum complexes with phosphoric acid.

Reagents of this character prepared in many ways and of varying compositions, have been proposed as qualitative and, by color comparison with standards of known composition, quantitative methods for detecting and estimating phenols and many other compounds. This type of test has been extensively used by biologists and others.

Wu (1920) made a study of these complexes and it has been shown that the color reactions are produced by reduction. The reagent prepared in various ways is very sensitive to mild reducing influences. The color reactions are not to be attributed, or at least only in small part to the colored oxidation products of the phenols. The reagent is therefore not specific for phenols or in fact for anything except a reducing influence. Ever since Folin and Denis (1912) proposed this reagent it has been widely employed.

Under the heading of the arsenic reagents there is described Guglielmelli's modification.

References

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|------------------------------------|-------------------------------------|
| Folin and Macallum (1912) | Scott (1921) |
| Folin and Denis (1912, 1915, 1916) | Bezssonoff (1921, 1922) |
| Johns and Jones (1918) | Levine and Burns (1922) |
| Benedict and Theis (1918) | Ellms, Marshall and Phillips (1922) |
| Folin and Wu (1919) | Rakestraw (1923) |
| Gortner and Holm (1920) | Henningsan (1923) |
| Wu (1920) | Goeffon and Nepveux (1923) |
| Levine (1920) | Cristol (1924) |
| Tisdall (1920) | Haas and Schlesinger (1924) |
| Chapman (1921) | Vorce (1925) |
| Donaldson (1921) | |

Uranium. Uranium salts form colored complexes with phenols. Siemssen (1912); Aloy and Laprade (1905); Aloy and Rabaut (1914).

Vanadium. Mandelin's reagent is a solution of a vanadate in concentrated sulfuric acid.

References

Mandelin (1884)
Lévy (1886)

Bellier (1899)
Parry (1924)

Zinc. Zinc chloride is employed as a condensing agent in producing the phenol phthalein colors. Zinc chloride and zinc sulfate are used by Nickel (1890) in connection with nitrous acid reagents.

D. Tests not classified in the foregoing

a. Acetylation. Acetylation of phenols has been employed in some cases for quantitative measurements. Verley and Bölsing (1902); van Urk (1921).

b. Alkaloidal precipitation. Hesse (1876) found that certain alkaloids formed precipitates with phenol and indicated that these might be employed as tests.

c. Urethan test. Herzog (1907) employed diphenylcarbamyl-chloride to produce diphenylurethans from phenols and French and Wirtel (1926) made use of alpha-naphthylisocyanate in the formation of urethans. A number of urethans were analyzed and characterized by their melting points.

d. Electrometric titration. Various phenols have been estimated by this method. Kolthoff (1920); Bishop, Kittredge and Hildebrand (1922).

e. Enzyme test. Bourquelot (1906) showed that *Russula delica* contained an enzyme that gave colors with a large number of phenols. Schmalfuss (1924) employed an enzyme from the blood of larvae of *acta caja* to differentiate between many phenols by means of the color formation. Onslow and Robinson (1925) found an enzyme in potato tubers that acted on phenols.

Mamas (1923) found a peroxidase in melanotic tumors that acted on polyphenols when two hydroxyl groups are in the ortho position to each other.

f. Esterification. Various esterification tests can be employed

to detect phenols. Hesse (1906) methylated isoborneol as a method of detection.

g. Hydrogen peroxide. Angelo (1922) obtained a fine rose or red color by oxidizing phenol with hydrogen peroxide in presence of diammonium phosphate or disodium arsenate. Without the addition of the salts the color development is much slower. For tests employing sodium peroxide see sodium. Wurster (1887); Kühl (1905).

h. Picrates. Goedike (1893) employed picric acid to precipitate various phenols; phenol, o-cresol, resorcinol, quinol, guaicol and other.

i. Wood test. Runge test. This test depends upon the formation of a blue color in a splinter of wood (usually pine) on treatment of the wood first with phenol and then with concentrated hydrochloric acid. With some phenols the color is red. This test is severely condemned by Khotinskii.

References

Runge (1834)
Dispensatory, U. S. (1870)
Waller (1881)

Tommasi and Tommasi (1881)
Udránszky (1888)
Khotinskii (1917)

DELICACY OF TESTS

Very few of the tests have any great degree of delicacy. The chlorine test as applied to potable waters, is claimed to be the most delicate and that even less than 1 part of phenol in 10,000,000 can be detected by the odor and taste.

Eykman claims 1:2,000,000 for the ethylnitrite test, and Wilkie 1:250,000 for the iodination test. The arsenotungstic, phosphotungstic-phosphomolybdic and the Millon reagents and the azo dye tests are stated to detect 1:1,000,000. The arylsulfonhalogenamides tests may be delicate in the case of β -naphthol, to 1:100,000 and the bromine test to 1:60,000. The ferric chloride test is not claimed to have a greater delicacy than 1:3,000 for phenol while for salicylic acid 1 part in 100,000 may be detected.

Various claims are made for some of the other tests, but it is

evident that the delicacy depends not only upon the conditions of the reagent and the solutions of the phenols, but also upon the character of the phenol present. The delicacy of a test that is more or less general for a number of phenols may vary greatly with the different phenols.

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THE USE OF X-RAYS FOR THE DISCOVERY OF NEW ELEMENTS¹

G. HEVESY

When Moseley started his researches on the spectroscopy of X-rays one of his immediate aims was to ascertain whether the regularity to be found would follow the sequence of the atomic weights or that of the atomic numbers, whether the sequence Fe, Ni, Co or Fe, Co, Ni would be found (1). The result of his efforts is well known, as well as the simple relation between wave length and atomic number which he discovered. Moseley entertained no doubt, that X-ray spectroscopy would prove to be of great use in the analysis of alloys and of minerals and specially in searching for missing elements. In fact, he carried out an investigation of a rare earth sample sent to him by Urbain which showed the optical "celtium lines." He could not detect the presence of other than known elements, namely those of the last members of the rare earth group. The later identification of the optical "celtium lines" with those of the element 71 (lutecium) in fairly pure state was in full accordance with Moseley's results. Apart from this single case, Moseley did not embark on chemical analysis but endeavoured to elucidate the K- and L-series of all the available elements. Moseley's work was continued by Siegbahn, and we owe to him and his collaborators great progress in elucidating the X-ray spectra of most of the chemical elements as well as the development of the technique of X-ray spectroscopy which made the X-ray method easily applicable to analytical purpose.

Working in Siegbahn's laboratory about eight years later Hadding (2) emphasized the use of X-ray analysis for the detection of elements in minerals and especially of the rare earth

¹ It is with very great pleasure the writer is following the request of the editor to write an article on the above topic.

elements, many of which are not distinguished by the usual analytical methods. Following up the suggestion of Hadding, who himself applied X-ray analysis to minerals containing rare earth elements, Goldschmidt and Thomassen (3) carried out a very exhaustive research and were thus enabled to give a rough estimate of the ratio of the rare earth elements present in practically every one of the more important rare earth minerals. We may add that the important results of Goldschmidt and Thomassen could hardly have been obtained in any other way. When Coster and the present writer (4) were faced at about the same time with the problem of detecting and estimating hafnium no other method could be used but X-ray analysis. They mixed a known amount of tantalum oxide with the sample to be investigated and measured the intensity of the corresponding tantalum and hafnium line, assuming that the effect of equivalent amounts of hafnium and tantalum on the photographic plate is about the same. Later when in possession of pure hafnium oxide this method was replaced by a somewhat different one (5). A known amount of the oxides of the elements 71 and 72 were mixed with ZrO_2 and the intensity ratio of the $\text{Lu}\beta_2$ and $\text{Hf}\beta_1$ lines determined at a known tube voltage. To estimate the hafnium content of a sample, the latter was turned into oxide and a known amount of Lu_2O_3 added. By comparing the intensities of the above mentioned two lines which lie very closely together on the photographic plate² and knowing the intensity ratio of the lines given by a sample containing equal quantities of HfO_2 and Lu_2O_3 , the amount of hafnium present can easily be calculated. As the problem of the exact estimation of the elements by the X-ray method lies beyond the scope of this report, we shall not discuss this or similar methods further.

GENERAL CONSIDERATIONS OF X-RAY SPECTRA

The question may be put why the introduction of X-ray spectroscopy is to be considered a highly remarkable and in

² These lines are only 4x units apart, a circumstance which is very favorable to an accurate comparison of their intensities.

some respects quite fundamental step forward in analytical chemistry. The answer to this question can be summed up in the following points:

I. As X-ray spectra are connected with the temporary removal of electrons from inner electronic groups, while the optical spectra are connected with the removal of outer electrons, the X-ray spectra are necessarily less complex than optical spectra.

II. The appearance of the X-ray lines is practically independent whether the element is present in the free state or as any compound. Optical spectra are highly dependent on the state of the element; furthermore the presence of other elements may partly or entirely suppress the appearance of some lines.

III. The position of the X-ray lines can be calculated in advance, a fact which is of great importance in searching for missing elements.

We may further add that the ease with which the tube voltage and thus the excitation of the X-lines can be regulated is of great practical importance, since it allows the excitation or suppression of a given line at will. Any chemical element which lies between sodium and uranium in the periodic table can be identified by its K-lines, any between copper and uranium by its L-spectrum. Lines belonging to these groups are the ones generally used in recent analytical work and only exceptionally the M-lines of the heaviest elements. The possibility of the coincidence of a K-line with other lines is in general appreciably smaller than this possibility for an L-line, and therefore the K-series is more useful for the identification of an element, especially, as we shall discuss more in detail later, if the element to be detected is present only at a low concentration. But on account of the much higher voltage, which the excitation of the K-series requires, the L-lines have hitherto been more frequently used for the determination of the heavier elements. We will in the following consider the detection of tantalum and discuss in connection with this example the general features and the possible errors of X-ray analysis.

THE DETECTION OF TANTALUM

To establish the presence of tantalum in a mineral we fix a small sample of the pulverised mineral on the anticathode and bombard this with cathode rays. L_{α_1} being the strongest line of the L-series, the presence of this line will be first sought for, using a vacuum spectrograph.* To excite the L_{α} line a minimum of 10,000 volts is necessary, but a higher one, for example 18,000 volts is more convenient. Let us assume, that on the photographic plate a line appears at the expected position 1518 X ($= 1,518 \text{ \AA}$) and ask whether the appearance of this line is conclusive evidence for the presence of tantalum. If the line is sufficiently strong and the experimenter sufficiently skilful to locate it with an accuracy of an X unit, we may answer in the affirmative. Should this not be the case the appearance of the line is far from being conclusive for the presence of tantalum. In the case of a faint line the error of measurement may amount to several X units and if an error of 3 X units is made the Dy L_{γ_5} line may be mistaken for the Ta L_{α_1} line. We may decide which line we have observed in either of two ways. We may abandon the α_1 line and try to excite the $\beta_1, \beta_2, \alpha_2$ and other lines or we may ascertain the absence of the Dy α_1 line; if the latter is absent, the much weaker Dy γ_5 line can not be present. The first mentioned alternative is in general to be preferred. The next strong tantalum line has the wave length 1324 X, a line exactly coinciding with hafnium β_2 and differing only by 3X units from $\text{Pt}\alpha_2$. Tantalum β_2 exactly coincides with $\text{Zn}\beta_2$ and comes very near to $\text{W}\beta_1$. If the sample contains 1 per cent or more of tantalum as well as the α_1 line, the α_2 line will be easily visible. The appearance of such a "doublet" composed of two lines of widely different intensities (10:1) is always reassuring for the X-ray spectrographer, though the possibility of queer coincidences may even in this case not quite be disregarded. But the expected intensity ratio of the lines is in general one of the most decisive proofs of the presence of the element sought for, considering that this constant ratio is known and very different for different rays.

* A vacuum is necessary since the soft L-rays are absorbed by air.

Besides the possibility of the coincidence of the Dy_{75} line with the $Ta_{\alpha 1}$ line the possibility of the coincidence of the second order $Bi L_{\gamma 4}$ with the latter must be faced. This possibility can be easily tested by lowering the voltage under 16,000 volts, the minimum voltage for the bismuth line. The use of very high voltages to produce the lines increases the intensity of lines, but increases at the same time the possibilities of coincidence of the line sought with secondary, tertiary and higher order of other elements so far as the L series is considered. When investigating the K line this disturbing effect of the use of very high voltages does not usually interfere, hence the advantages of the use of the K lines when the presence of very small amounts of an element has to be ascertained.

The behavior of tantalum as regards coincidence of its lines with those of other elements is by no means exceptional and the above example may suffice to show the unsafe ground on which we are moving when trying to identify an element on the basis of few very faint lines, as some investigators (6) claim to have done. We must further remember that such faint lines can not only be due to coincidence but can also be produced through an imperfection of the diffracting crystal. Besides the emission lines also the absorption edges have repeatedly been used to identify elements (7). The appearance of the K absorption edge at 183, 6 X is, for example, strictly characteristic for the presence of tantalum.

We will discuss in the following the use of X-ray analysis for discovery of missing elements.

THE ELEMENT 72

When searching for the element 72 in zirconium minerals Coster and the writer could see on the first plate exposed the $L_{\alpha 1}$ line for which they were searching (comp. figure 1). As the mineral investigated contained so much as about 2 per cent hafnium silicate the line was very distinct as may be seen on figure 1 which represents a photometric curve of the blackening of the photographic plate. To make the result obtained conclusive the investiga-

tion was continued simultaneously in two different directions. The attempt was made to excite the other lines of the L spectrum calculated by interpolation and to investigate the effect of chemical treatment on the intensity of the α_1 line, i.e., on the concentration of the element in the sample emitting this line. The mineral was fused with acid potassium fluoride and the melt

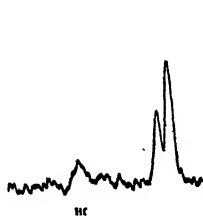


FIG. 1

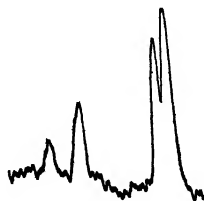


FIG. 2

FIG. 1. PHOTOMETRIC CURVE OF THE FIRST ZIRCON INVESTIGATION
FIG. 2. PHOTOMETRIC CURVE AFTER THE FIRST CHEMICAL CONCENTRATION OF HAFNIUM

extracted with hot water containing some hydrofluoric acid and crystallized a few times. The intensity of both the α_1 and the α_2 line increased very markedly as in the spectrum of the mother liquor obtained by this process shown by figure 2, thus clearly indicating that these lines are due to a substance which can be separated from zirconium.

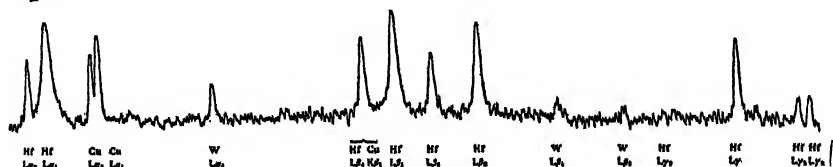


FIG. 3. PHOTOMETRIC CURVE OF THE X-RAY SPECTRUM OF PURE HAFNIUM

The X-ray method was not only found to be a unique analytical weapon in the discovery of the element 72, but also in the process of isolating hafnium, when the method was employed to check the result of every step of the prolonged process of separation. The complete L spectrum of hafnium taken by Coster (8) is shown by figure 3. It was obtained by using practically pure HfO_2 . It is of interest to note the rather peculiar coincidence

of the L_{α} doublet of hafnium with the second order K_{α} doublet of zirconium. This coincidence is somewhat disturbing, as hafnium always occurs together with zirconium. When using a voltage exceeding 18,000 we cannot distinguish the hafnium lines from zirconium lines. By lowering the voltage we can, however, suppress the ZrK_{α} doublet.

THE ELEMENTS 43 AND 75

While hafnium is sufficiently abundant to be detected by the X-ray method, in any mineral containing zirconium, elaborate trials to detect the presence of the apparently very rare elements 43 and 75 in minerals entirely failed. In 1925 Noddack, Tacke and Berg (9) announced the discovery of these elements in chemical preparations produced by very elaborate work from columbite and various other minerals and proposed for these elements the names of masurium and rhenium respectively. They estimate that the minute samples obtained contained a small per cent of the elements looked for. The following lines were observed:

	43			75				
	K_{α_1}	K_{α_2}	K_{β_1}	L_{α_1}	L_{α_2}	L_{β_1}	L_{β_2}	L_{β_3}
Observed.....	672.	675.	601.	1429.9	1440.7	1235.0	1204.8	1216.0
Calculated.....	673.4	677.9	600.0	1430.6	1440.6	1235.5	1204.1	1216.9

Let us now consider the possible coincidences of other lines with lines of the element 75. The L_{α_1} line of 75 coincides with $Zn K_{\alpha_2}$, but $Zn K_{\alpha_2}$ is appreciably weaker than $Zn K_{\alpha_1}$, whilst the $75\alpha_1$ line is about of the same intensity as the $Zn K_{\alpha_1}$ line, also seen on the plate. The very weak L_{α_2} line of 75 does not coincide with any known line. The L_{β_1} line coincides with $W L_{\beta_3}$ but is, as Berg (10) remarks, stronger than we should expect it to appear from the intensity of the stronger tungsten lines seen on the plate. L_{β_2} coincides with $Tl\alpha_1$; L_{β_3} with $Tl\alpha_2$. Berg emphasizes that the intensity ratio of the lines observed is the expected one. Later Heyrowsky and Dolejssek (11) reported the appearance of the L_{α_1} , L_{β_1} , L_{β_2} and L_{γ_2} of the element 75 in preparations

extracted from "pure" manganese compounds. The results of the X-ray examinations quoted above have not yet been corroborated by any chemical or optical evidence. We have also to mention, that similar experiments as the above described ones were recently carried out partly by Zvjaginstsev (12) and partly by Prandtl (13), both with negative result.

THE ELEMENT 61

In the case of the element 61, the last missing rare earth element, optical spectroscopy proved to be more sensitive than the X-ray method. It was with the aid of the former that Harris, Yntema and Hopkins (14) succeeded first in showing the presence of the element 61 (illinium) in fractions of rare earth preparations obtained after very elaborate chemical work. They were actually guided in their work by the observation of the absorption band spectra, but succeeded in showing the presence of a very faint L_{α_1} and L_{β_1} line in their most concentrated preparation after a very long exposure and intense bombardment of their sample with cathode rays. Simultaneously with Harris, Yntema and Hopkins, R. J. Meyer, Schumacher and Kotowski (15) were also successful in concentrating illinium sufficiently for it to be shown by an X-ray examination made by Dehlinger, Glocker and Kaup (16). In the latter investigation the use of the K-lines proved to be of great value, as very high voltages could be applied without the risk of coincidence of the lines observed with any line of higher order and because the possibility of a coincidence of primary lines in this short-wave region of the spectrum is much smaller than in the region of the L spectrum. One of the K lines of illinium, 61 K_{β} , coincides, however, with TbK_{α_2} . The L lines of the element 61 coincide almost exactly with the following lines. $Il_{\alpha_1} + \alpha_2$ with La_{β_2} and Bi_{α_1} (second order); $Il_{\beta_1} + \beta_4$ with Pr_{β_7} ; Il_{β_2} with Sm_{β_3} ; Il_{γ_1} with Nd_{γ_2} , and so on. It is of interest to note that Meyer and his collaborators used the same method to concentrate 61 (James' bromate crystallization) as did Harris, Yntema and Hopkins. A somewhat different method was used

by Rolla and Fernandes (17). These investigations were carried out simultaneously with those previously mentioned and resulted in obtaining a concentration of element 61 sufficient to give optical absorption bands which are considered characteristic of this element as well as a very faint K absorption limit in the X-ray spectrum.

As to the two missing elements in the region between hydrogen and uranium, the elements 85 and 87, the general evidence tends to show that only radioactive isotopes of these elements are likely to be found.

A detailed description of the method of X-ray analysis and an exhaustive table of the known X-ray lines is to be found in the brilliant monograph of Siegbahn "The spectroscopy of X-rays," Oxford University Press 1925.

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THE PRESENT STATUS OF TURBIDITY MEASUREMENTS

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New York City*

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INTRODUCTION

Every attack on the problems of dispersed systems is disappointing, because of the baffling complexity of the phenomena. One optical method after another has been eagerly snatched, applied, and often abandoned after trial. Diaphanometers, nephelometers, turbidimeters, tyndallmeters, dispersimeters, opacimeters, have been developed and placed on the market, but not one has yet been accepted as a standard instrument for the laboratory. To read the long list of new applications one would expect by now to find such an instrument in every analytical laboratory. Apparently turbidity measurements have not proven satisfactory, and yet the prospects are more hopeful

than they seem. Once the limitations of such optical methods are understood, their real possibilities will be appreciated for what they are worth.

Turbidity measurements have the fundamental advantage of revealing properties of the dispersed phase without disturbing its delicate equilibrium. Light vibrations are so gentle that even vital processes are not seriously upset by their passage. It is, therefore, possible to measure the phenomena optically while they are taking place. Gravimetric analysis sacrifices the sample. Moreover, some dispersed phases cannot be separated from the dispersing medium, while others require much time and effort for the separation. Turbidity can be measured without sacrificing the sample, with little time and effort. The optical criterion is extremely sensitive, having been applied with success to measure the light scattered by gas molecules. Needless to say, then, that it surpasses the microbalance in sensitivity. It must not be forgotten, also, that turbidity is a measure of other factors beside concentration. When concentration only is desired this is a disadvantage, but there is good reason to expect that the other variables will soon be of interest also. Such considerations justify a much more serious study of turbidity measurements than they have been given heretofore. In this review are presented briefly the theoretical basis of turbidity measurements and an estimate of the quality of the instruments that have been devised to measure turbidity.

I. SOLUTIONS AND DISPERSIONS

Simple fluids are supposed to consist of but one species of molecule, although some phenomena indicate that even water is much more complex. Solutions, also, are molecularly dispersed, not more than a few molecules at a time associating as aggregates. Even at the surface of solutions, where the molecular structure is different from that in mass, the transition layer is not many molecules thick. When an insoluble substance precipitates from a solution, however, large aggregates of molecules condense about each nucleus forming a dispersed phase. The

natural tendency for such aggregates is to combine into still larger ones until the particles attain microscopic dimensions, but in many cases this growth is arrested, leaving a vast number of very small particles presenting a large surface to the solution.

If the dispersed phase is solid the dispersed system is usually called a suspension, if liquid, an emulsion, although "photographic emulsions" consist of solid silver halide grains dispersed in gelatin. Liquid drops dispersed in vapors are called mists, clouds, fog, or smokes. When the particles are submicroscopic, the colloid is called a sol, when coagulated, a gel, etc. Dr. Exton has suggested calling dispersed systems in general *dispersions*, to avoid the unfortunate term *dispersoids*, which is sometimes used. The important characteristic of dispersions is the large rôle played in them by surface phenomena. Cutting a centimeter cube into little cubes one micron on each edge increases the surface ten thousand fold, spread over a million million particles. Over one hundred million silica particles per cubic centimeter, each a tenth micron in diameter, are required to produce even so small a concentration as one milligram per liter, or as it is commonly expressed, one part per million. The red corpuscles present over 400 times as much surface as a cubic centimeter of blood, yet they are several microns in diameter. Think of what an enormous interface is presented by the sub-microscopic dispersed phase in a gel!

Dispersions are not in true thermodynamic equilibrium, according to Tolman (1),¹ until the particles are all of the same size and the surface tension between the particles and the medium is zero. But most dispersions are found to contain particles widely distributed in size, so that the tendency toward equilibrium must be resisted by protective membranes or adsorbed layers. Many protein precipitates in the Prudential Laboratory remain dispersed for years. Although the precipitate flocculates easily, and settles to the bottom of the vessel, a slight agitation is sufficient to separate the flocculi again into microscopic particles. Surely in such cases there must be a very positive

¹ The number in parentheses indicates the paper referred to in the bibliography.

repulsion between the particles which prevents them from coalescing. The smaller particles are in incessant Brownian motion, or thermal agitation, and are therefore continually colliding. While in contact, the particles tend to coalesce and thereby reduce the surface energy, so that to remain separate this tendency must be opposed by repulsive forces between the transition layers. It seems to be the irreversibility of the process of formation of these surface layers which accounts for the comparative stability of many dispersions.

Condensation nuclei

The phenomena of supersaturation show that nuclei are always required to initiate the condensation of a dispersed phase. Very little is known about condensation nuclei except those for water vapor, which have been studied extensively during the last fifty years. The main interest of the earlier investigators, Forbes, Coulier, Mascart, Aitken, Kiessling, and the younger Helmholtz, was in understanding the varied phenomena of the atmosphere, but the later work of Barus, Lenard and Ramsauer, Townsend, J. J. Thomson, and C. T. R. Wilson has led directly to our modern knowledge of the constitution of the atom. One reason for this has been that water vapor is an ideal medium for studying condensation phenomena. It can be supersaturated simply by sudden expansion, and although the drops soon evaporate again, the condensation nuclei can be studied easily in great detail. The classic results with water vapor are fundamental in the study of dispersions.

Lord Kelvin, in 1862, showed that the effect of surface tension alone required a certain degree of supersaturation in the vapor about small particles for thermodynamic equilibrium. The supersaturation increases exponentially as the radius of the particle decreases. If the particle is electrically charged the supersaturation is depressed. The same effect results if the particle has a specific attraction for water, forming a concentrated solution nucleus with low vapor pressure. Hygroscopic substances can thus induce "incipient condensation" at humidities

below 100 per cent, as observed by Aitken in 1880. The thick haze of hot weather is due to this incipient condensation.

The colors of the steam jet have provided a measurement of the size of the millions of condensation nuclei present in every cubic centimeter of ordinary air, but the method of repeated sudden expansion under controlled conditions, as perfected by C. T. R. Wilson (1) in England and Barus (1) in this country, provides a much more powerful instrument of research. If we take ordinary room air and enclose it in a vessel containing some water long enough to become saturated, and then expand it suddenly by raising a piston, a fog will form throughout the air and water will condense on the surfaces of the vessel. If we allow the fog to settle, and the air to become again saturated, another expansion produces another fog. But after repeating this several times, the fog becomes thinner each time, the drops larger as they become fewer, until finally no drops are formed on slight expansion. There are evidently present in the air particles upon which the water vapor condenses, and which settle out in the drops. These are the persistent nuclei, and the water vapor condenses throughout the air only when these are present. Air filtered slowly through cotton wool is free from such nuclei, which range in size from dust particles to the large ions, mere clusters of molecules.

Myriads of persistent nuclei are produced in combustion, evaporate from many chemicals such as phosphorus, result from the bursting of bubbles on solutions, from electrical discharges and from ultraviolet light, x-rays, radium, etc. In addition to these large nuclei are the small ions which are being always continuously produced even in a closed container at a definite rate, and which require above a four-fold supersaturation to induce condensation. Most remarkable of all, at supersaturations above 8, billions of nuclei per cc. are spontaneously produced from the vapor itself, the resulting dense fogs showing large green-centered coronas which are beyond the range of optical theory. Here we are obviously entering the region of molecular kinetics, where the statistical distribution is reproduced by the conditions of thermal equilibrium.

In other vapors the phenomena are similar so that we are led by analogy to believe that precipitation in solutions is governed by the number of nuclei effective at a given supersaturation. Indeed, Von Weimarn (1) has surveyed the laws of precipitation of barium sulfate over a vast range of supersaturations, and finds the same general increase in number of nuclei and fineness of particle with the supersaturation. It must be remembered, however, that increasing the supersaturation in itself produces larger particles when the number of nuclei remains constant, for there is more mass to condense. It is the successive inclusion of new classes of nuclei, smaller in size but vastly greater in number, which distributes the condensing mass into finer aggregates. Once the condensation begins, the number of nuclei is fixed, for the condensation process relieves the supersaturation. Those nuclei which have not become effective at the peak of the supersaturation are still less efficient while it is falling. Of course it often happens that the diffusion process is so slow that wide variations in the supersaturation occur locally. In such cases the precipitation is extremely complicated, and difficult to reproduce.

Precipitation from solutions

First, consider the case where the condensation is regulated by the diffusion process. This requires the simultaneous existence throughout the process of the two reacting phases, with a diffusion layer between presenting all stages of transition in concentration. It is therefore obvious that in this case it makes no difference which phase is added to the other, for the conditions are symmetrical, each phase being present in excess on its side of the transition layer.

At the instant of contact of the two phases, a sharp gradient exists in the concentration, which is gradually wiped out by diffusion, the transition layer increasing in thickness until the supersaturation somewhere in the layer becomes sufficient for precipitation to occur upon the nuclei. The state of affairs at this instant is schematically presented in figure 1, where the ordinates (concentrations) are plotted against the distance

across the transition layer. The scales, of course, are all out of proportion. Next to the pure phase of concentration *A* is a layer of excess of *A*, the supersaturation of *BA* increasing as the excess of *A* decreases, until a layer is reached where there is an excess of *B*, where the supersaturation of *BA* decreases as the pure phase of concentration *B* is approached. It is evident that the nuclei in the different regions of the diffusion layer are in quite different environments. Precipitation begins where the

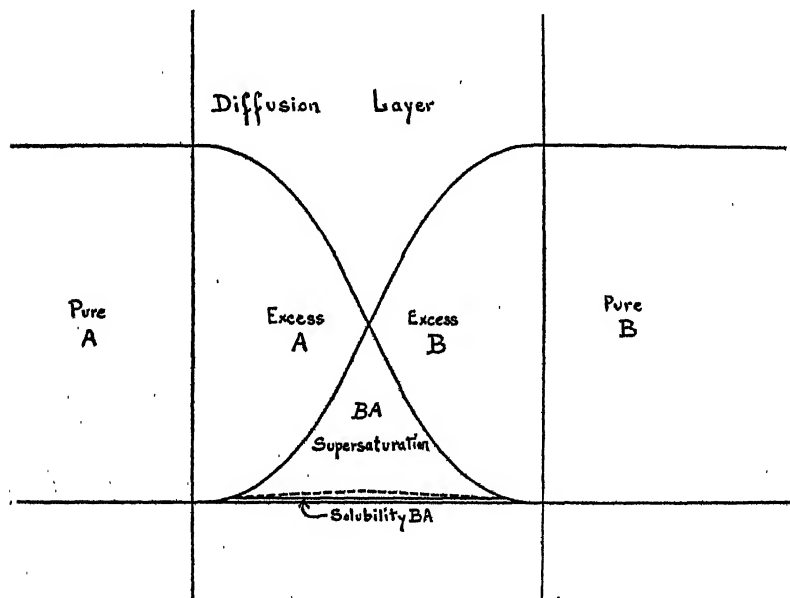


FIG. 1. CONCENTRATION RELATIONS AT START OF PRECIPITATION

particular local concentration environment is sufficient to induce condensation on the nuclei present. Since the solubility of *BA* is constant throughout the layer, the solubility of each component is reciprocally as the excess of the other, and this is shown by the dotted line representing the soluble portion of the minor component. The concentration of *BA* available for precipitation is given by the portion of the ordinate between the dotted line and the total concentration of the lesser component. As soon as condensation begins, however, the whole picture changes with

explosive rapidity. The supersaturation about the particles practically vanishes, and this prevents any more diffusion of the minor component beyond this zone, while the diffusion to this zone, of the particular components which are condensing into the dispersed phase, is accelerated. In other words, the pure phases must penetrate a sort of "no man's land," where a large proportion of the reactants pass out into the dispersed phase, before they can diffuse into each other.

Some observers have tried to explain the increase in turbidity after precipitation begins as an increase in the number of particles. This seems improbable, for after the most efficient nuclei have induced condensation upon them, only the less efficient nuclei are left, and these could not compete with the larger particles for much of the precipitate. Indeed, the larger particles are more apt to rob the smaller ones, as indicated by Kelvin's formula, so that an actual decrease in the number of particles is more likely to occur. The coalescence of particles on colliding would also diminish the number. One way in which new particles could form would appear to be when the particles already present become poisoned by adsorbed layers at a certain stage of their growth; but in such a case it is hard to understand why the smaller nuclei are not also poisoned by the same adsorbed layers.

The only way in which the precipitation can spread from the thin layer where it starts is by the diffusion of the particles away from the locality. Diffusion is merely the result of thermal agitation (Brownian movement). This may sometimes leave the field open for condensation to start afresh upon other nuclei, but the occurrence of particles of different sizes is more probably due to the varying rate of precipitation as the particles diffuse into regions where the supersaturation is different. A still more potent source of variation is the adsorption of impurities on the surfaces of the particles, which may occur at different stages of their growth, and poison further condensation. It is only when the same supersaturation occurs simultaneously throughout a uniform solution, brought about for example by lowering the temperature, that one would expect to obtain uniform particles.

In many cases of precipitation it makes considerable difference

which phase is added to the other. The process is evidently irreversible. If the mixing is complete before precipitation occurs, the excess of the major component never falls below its final value during the addition, if the minor phase is added to the phase in excess. But if the mixing is much more rapid than the condensation, it should be possible to complete the addition before appreciable precipitation, and what occurs thereafter does not depend on which phase is added to the other. Since the dissymmetry cannot occur while both pure phases are present, the minor phase must be exhausted as fast as it is added. Under such conditions there is the minimum possibility of precipitation in an excess of the minor phase, and any particles which might form in such an environment pass into an excess of the major phase after the shortest time. The formation of the colloid particle is lucidly described by Svedberg (2).

Law of particle growth

When the surface of the particles is so large that the condensation is limited by diffusion, we have the rate of increase of the concentration (C) of the dispersed phase

$$\frac{dC}{dt} = k(C_m - C) \quad (1)$$

where C_m and k are constants. For N spherical particles of uniform diameter d , $C = kNd^3$, and assuming N to remain constant, the integral of (1) gives the diffusion law of particle growth

$$kt = \log(1 - x^3) \quad (2)$$

where x is the ratio of the diameter at the instant t , to the final diameter when all the insoluble material (C_m) has precipitated out. The constant of integration, $\log(1 - x_0^3)$ can be neglected, and of course the k 's are different constants in the different formulas.

When the surface of the particles is also a limiting factor, equation (1) must be modified to

$$\frac{dc}{dt} = kNd^2(C_m - C) \quad (3)$$

which is equivalent to the equation

$$\frac{dx}{dt} = k(1 - x^3) \quad (4)$$

with the same assumptions as above. The integral of (4) gives the surface law of particle growth

$$kt = F(x) - F(x_0) \quad (5)$$

where

$$F(x) \equiv \log \left[\frac{1 + x + x^2}{(1 - x)^3} \right] + 2\sqrt{3} \tan^{-1} \left(\frac{2x + 1}{\sqrt{3}} \right) \quad (6)$$

and x_0 is the initial value of the diameter ratio.

TABLE 1

	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.99	1
$F(x) - F(x_0) \dots \dots$	0.602	1.205	1.811	2.439	3.103	3.813	4.653	5.707	7.32	12.07	∞

Two factors have been ignored in the above theory, (1) the decrease in the number of particles due to coalescence, (2) the poisoning of their surface for condensation by adsorbed layers. The frequency of collisions which must precede coalescence, is proportional to the number of particles per cubic centimeter and to the rate of diffusion, which is inversely proportional to the particle diameter (assuming the viscous resistance law of Stokes). Hence

$$-\frac{dN}{dt} = k \frac{N}{d} \quad (7)$$

which is equivalent to

$$d\chi = kdt \quad (8)$$

when the concentration of the dispersed phase is fixed. The integral of this equation gives a linear relation for the coalescence law of growth,

$$kt = d - d_0 \quad (9)$$

where d_0 is the initial diameter (of the nuclei).

The adsorption of impurities probably follows Freundlich's equation, but when coalescence and adsorption are combined with the surface law an integral equation is obtained which we shall not attempt to solve. The crude theory given above will be considered later in the interpretation of the growth of turbidity. A tabulation of the function (6) is given in table 1, for a few values of χ . An interpolation plot on logarithmic paper is most convenient in testing the equation.

II. OPTICS OF DISPERSIONS

Perfect diffusion

The complete theory of the behavior of scattering media in fully diffused light has been worked out by Channon, Renwick, and Storr (1). If I_o be the incident light intensity, I_r the intensity rejected by the diffusing layer, and I_t that transmitted, the rejectance R is defined by the ratio I_r/I_o , and the obstructance Ω by the ratio I_o/I_t . Superposing two layers (m) and (n), the intensity transmitted through them both, taking account of the successive inter-rejections, is

$$\frac{I_o}{\Omega_m \Omega_n} (1 + R_m R_n + R_m^2 R_n^2 + R_m^3 R_n^3 + \dots) = \frac{I_o}{\Omega_m \Omega_n (1 - R_m R_n)}$$

so that the combined obstructance is

$$\Omega_{m+n} = \Omega_m \Omega_n (1 - R_m R_n) \quad (10)$$

Since this is symmetrical, the obstructance is independent of the arrangement. The combined rejectance is similarly

$$R_{m+n} = R_m + R_n / \Omega_m^2 (1 - R_m R_n) \quad (11)$$

The relative obstructance (O) of a layer superposed on a standard of rejectance ρ , is defined as $O \equiv \Omega(1 - R\rho)$, so that the proper expression for the diffuse density is

$$D \equiv \log O = \log \Omega + \log (1 - R\rho) \quad (12)$$

where \log is the symbol for the logarithm to the base 10. When the rejectance of the sample is very small, as in the black silver

deposit of photographic images, the rejectance factor can be neglected. The density is evidently never as great as it would be without the rejectance factor.

The general expression for the obstructance as a function of the thickness (χ) is

$$O_x = P \exp(\alpha\chi) + (1 - P) \exp(-\alpha\chi) \quad (13)$$

where P and α are constants, and \exp is the symbol for the exponential function. This is evidently the integral of $d^2O/d\chi^2 = \alpha^2 O$. Schuster (1) has derived a similar equation for the radiation through a foggy atmosphere. The relation for Ω_x is of

TABLE 2

OPAL GLASS THICKNESS (χ)	DENSITIES (AIR CONTACT)			DENSITIES (OIL CONTACT)		
	D_χ (approx.)	$\log O_\chi$ (exact)	Diff.	D_χ (approx.)	$\log O_\chi$ (exact)	Diff.
<i>mm.</i>						
1	0.282	Used for	0	0.270	Used for	0
2	0.492	constants	0	0.490	constants	0
3	0.680	0.675	-0.005	0.694	0.692	-0.002
4	0.856	0.848	-0.008	0.889	0.887	-0.002
5	1.023	1.015	-0.008	1.076	1.080	-0.004
6	1.185	1.180	-0.005	1.260	1.271	-0.011
7	1.340	1.344	-0.004	1.438	1.462	-0.024

precisely the same form. It is evident from the form of this expression that the density increases more slowly than the thickness, and indeed Bloch and Renwick (1) have found an approximate form for the density

$$D_x = D_1 \chi^n \quad (14)$$

to hold within the limits of experimental error over a five-fold range in thickness. The exponent for the opal glass samples measured was $n = 0.80$ with air contact, and 0.86 with cedar oil contact. When the thickness was expressed in millimeters, the value of P was 1.342 and $\alpha = 0.4392$, for opal glass with oil contact, while for air contact $P = 1.600$ and $\alpha = 0.3838$. The agreement between the approximate and the exact equations is shown in table 2.

The densities calculated from the approximate equation never differed from those observed by more than 0.007, and 0.0023 was the average difference. This agreement is equivalent to a photometric precision of 0.5 per cent, quite up to the best standards of photometry. Richtmyer and Crittenden (1) give for the average deviation of a single observation from the mean of its set, 0.3 per cent, in a study of the photometric precision of 20000 readings by 15 observers.

Their general expression for the rejectance as a function of the thickness is

$$R_x = R_{\infty} [1 - \exp(-2\alpha x)] / [1 - R_{\infty}^2 \exp(-2\alpha x)] \quad (15)$$

where R_{∞} is the rejectance of an infinitely thick layer. Their measurements on opal glass gave 91 per cent for the maximum total rejectance of a thick solid block, which checks the values obtained on similar materials by A. H. Taylor (1) with his diffuse reflectometer, by an entirely different method. The laws of perfect diffusion, here briefly outlined, are therefore well established by this work of Channon, Renwick and Storr. Notice that the theory is purely geometrical, and makes no assumptions except that the light is perfectly diffused, and that the diffusing media are large enough to neglect edge effects. It has nothing to do with the mechanism of scattering by the particles constituting the dispersed phase.

The most important consequence of the laws of perfect diffusion for turbidity measurements is that the density increases more slowly than the depth. It disposes once for all of the claim sometimes made that one instrument is better than another because it conforms to the "theoretical" linear relation between density and depth. If the calibration curve of an instrument is linear, which of course is an advantage, it can be due only to some empirical shape factor which compensates for the rejectance factor in equation (12).

We may apply the laws of perfect diffusion to obtain a complete expression for the intensity of a thick Tyndall beam, as used in tyndallmeters and nephelometers. The incident beam is assumed to be of uniform intensity, and so each layer scatters

at right angles an increment which is proportional to its thickness. Before reaching the eye, this increment must traverse the depth (χ) of the dispersion, suffering an obstructance Ω_x . Moreover, the rejectances of the layers both above and below contribute their quota to the Tyndall intensity ratio T , and so the total increment (dT) due to the layer ($d\chi$) is given by

$$\frac{dT}{d\chi} = \frac{k}{\Omega_x} \frac{(1 + R_{a-\chi})}{(1 - R_x R_{a-\chi})} \quad (16)$$

where R_x is the rejectance of the layer above, $R_{a-\chi}$ is the rejectance of the layer below $d\chi$, and k is a constant. Substituting the functions of χ from (13) and (15) and integrating

$$T = \frac{T_\infty}{1 - R_\infty^2 u^2} \left[1 - Au - Bu^2 + (E - Fu^2) \ln \left(\frac{c+u}{c-u} \right) \right]^F \quad (17)$$

where $u \equiv \exp(-\alpha\chi)$, and A, B, E, F, c, T_∞ , and R_∞ are constants, and χ now represents the depth of the Tyndall beam. It is quite obvious from (17) that tyndallmeters and nephelometers give calibration curves even more complicated than do turbidimeters which measure the density of a dispersion. It is only when the concentration of dispersed phase is so small that secondary scattering can be neglected, or when empirical shape factors compensate, with dispersions of a certain range of concentration, that anything like a linear calibration curve is obtained. This limitation must be carefully investigated for every instrument before the results can be interpreted.

The Tyndall phenomenon

The beautiful experiments of Tyndall, which established the real cause of the color and polarization of sky-light, are fundamental in the optics of dispersions. A brief quotation from his papers follows.

The apparatus with which I work consists, as already stated, of a glass tube about a yard in length, and from $2\frac{1}{2}$ to 3 inches internal diameter. The vapour to be examined is introduced into this tube in the manner described in my last abstract, and upon it the condensed

beam of the electric lamp is permitted to act until the neutrality or the activity of the substance has been declared.

It has hitherto been my aim to render the chemical action of light upon vapours *visible*. For this purpose substances have been chosen, *one*, at least of whose products of decomposition under light shall have a boiling-point so high that as soon as the substance is formed it shall be *precipitated*. By graduating the quantity of the vapour, this precipitation may be rendered of any degree of fineness, forming particles distinguishable by the naked eye, or particles which are probably far beyond the reach of our highest microscopic powers.

I have no reason to doubt that particles may be thus obtained whose diameters constitute but a very small fraction of the length of a wave of violet light.

In all cases when the vapours of the liquids employed are sufficiently attenuated, no matter what the liquid may be, the visible action commences with the formation of a *blue cloud*. I would guard myself at the outset against all misconception as to the use of this term. The blue cloud here referred to is totally invisible in ordinary daylight. To be seen, it requires to be surrounded by darkness, *it only* being illuminated by a powerful beam of light. This blue cloud differs in many important particulars from the finest ordinary clouds, and might justly have assigned to it an intermediate position between these clouds and true cloudless vapour. . . .

In all cases, and with all substances, the cloud formed at the commencement, when the precipitated particles are sufficiently fine, is *blue*, and it can be made to display a colour rivalling that of the purest Italian sky. In all cases, moreover, this fine blue cloud polarizes *perfectly* the beam which illuminates it, the direction of polarization enclosing angle of 90° with the axis of the illuminating beam.

It is exceedingly interesting to observe both the perfection and the decay of this polarization. For ten or fifteen minutes after its first appearance the light from a vividly illuminated incipient cloud, looked at horizontally, is absolutely quenched by a Nicol's prism with its longer diagonal vertical. But as the sky-blue is gradually rendered impure by the introduction of particles of too large a size, in other words, as *real* clouds begin to be formed, the polarization begins to deteriorate, a portion of the light passing through the prism in all its positions. It is worthy of note that for some time after the cessation of perfect polarization the *residual* light which passes, when the Nicol is in its position of minimum transmission, is of a gorgeous blue, the whiter light of the

light of the cloud being extinguished.* When the cloud texture has be-

* This seems to prove that particles too large to polarize the blue, polarize perfectly light of lower refrangibility.

come sufficiently coarse to approximate to that of ordinary clouds, the rotation of the Nicol ceases to have any sensible effect on the quality of the light discharged normally.

Rayleigh's law

The theory of the scattering of light by small particles was given by Lord Rayleigh (1) in 1871.

The simplest case is that of a single particle of infinitesimal size compared with the wave length of light. The particle then acts as an electric oscillator, performing forced vibrations in the direction of the impressed force with a certain amplitude, a . The oscillator, therefore, sends out scattered waves in all directions, the vibrations being, of course, in every case perpendicular to the direction of the light, since light waves are transverse. But the component of a normal to a line making an angle θ with the vibration is $a' = a \sin \theta$, so that the scattered intensity in this direction, measured by the square of the amplitude is

$$I_s = ka'^2 = ka^2 \sin^2 \theta \quad (18)$$

Here the incident light is regarded as plane polarized. By (18) the scattered intensity vanishes when $\theta = 0$, that is normally to the incident ray, and in the direction of the incident vibration, in agreement with Tyndall's experiment.

If the light is unpolarized it is more convenient to consider, not the direction of vibration, but the direction of light propagation. If unpolarized light is incident along the axis of y , the incident vibration may be regarded as compounded of two vibrations of equal amplitude in the directions of the axes of x and z . If the particle is situated at the origin of coordinates, two vibrations of equal amplitude— a , along x and z —spread out in all directions from the origin as from a source. The components of these vibrations perpendicular to a direction r , defined by the angles α, β, γ , with the axes of x, y, z , are, respectively, $a \sin \alpha$ and a

$\sin \gamma$. The resultant intensity I_s , of the scattered light along r , is

$$I_s = ka^2 (\sin^2 \alpha + \sin^2 \gamma) \quad (19)$$

but from geometry $\cos^2 \alpha + \cos^2 \beta + \cos^2 \gamma = 1$, and hence

$$I_s = ka^2 (1 + \cos^2 \beta) \quad (20)$$

This gives the variation of the scattered intensity with the angle between the directions of the incident and scattered light. The intensity is a maximum in the direction of the incident light, decreasing to one-half normally, and zero in the opposite direction.

The amplitude of the vibration in the scattered light, on either the elastic solid theory or the electromagnetic theory of light, is proportional to the volume, V , of the small disturbing particle. At a distance r from the particle the amplitude must be inversely proportional to r , so that in order to be dimensionally correct, the ratio of the amplitudes a of the scattered light and a_0 , of the incident light of wave length λ must be

$$a/a_0 = kV/\lambda^2 r \quad (21)$$

These simple considerations may help to explain Rayleigh's expression for the intensity I_s of the light scattered from N particles each of volume V , the incident intensity being I_0 , and the wave length λ . This is

$$\frac{I_s}{I_0} = \frac{n'^2 - n^2}{n^2} \cdot \frac{NV^2}{\lambda^4 r^2} (1 + \cos^2 \beta) \quad (22)$$

Here n is the refractive index of the medium, n' that of the particles. The particles are supposed to be contained in such a small volume that the distance r and the angle β between the scattered and incident beams are the same for all the particles. For particles of different size, all small compared with the wave length, a summation must be made, requiring the size distribution of the particles. When the particles are not small compared with the wave length, terms of higher order must be included, and again the expression becomes complicated.

No account is taken in (22) of secondary scattering. All of

the light scattered by the particles in the direction β is supposed to reach the eye without loss. When the medium is densely filled with particles, this factor may become of first importance. The fractional decrease of the intensity I in traversing a thickness, $d\chi$, of the turbid medium is

$$dI/d\chi = -hI/\lambda^4 \quad (23)$$

where h is a constant independent of λ .
Integrating

$$I_\chi = I_0 \exp(-h\chi/\lambda^4) \quad (24)$$

where I_0 is the intensity of the light when $\chi=0$ and I_χ is the intensity after traversing a thickness χ .

The most striking characteristic of equations (22) and (24) is the occurrence of the factor $1/\lambda^4$, indicating that the scattered light increases rapidly as the wave length decreases. The scattered light is, therefore, much bluer than the incident light, while the blue is correspondingly absent in the transmitted portion, which contains a relatively large fraction of the red light. This was used by Rayleigh to explain the blue color of the sky, as well as the red colors of the sunset. The blue color may be used as a test of the size of the particles of any turbid medium. Thus the fine blue smoke from the end of a cigar is an indication that the smoke particles are much smaller than the wave length of light. Tyndall's test of complete polarization at right angles to the incident beam is still more sensitive.

The constant h in equation (24) has been evaluated by Schuster (2) from general considerations, independent of any particular theory of the mechanism of scattering. He obtains the same expression as did Lord Rayleigh, using the electromagnetic theory, namely,

$$h = 32\pi^2 (n - 1)^2/3N \quad (25)$$

where n is the refractive index of the dispersion as a whole. If the blue color of the sky is due to air molecules alone, N is Avogadro's constant, and its value is known from other measurements. The experimental researches on the absorption of the

atmosphere by Abbot and Fowle give good values for Avogadro's constant, and indicate quite definitely that the extinction coefficient is inversely proportional to the 3.9 power of the wave length, which is quite close to the inverse fourth power of Rayleigh's formula. Crova, Zettwich, and Boutaric have obtained inverse powers approaching, and in some cases even surpassing 4. Boutaric thinks that such excessive powers disagree with Rayleigh's theory, and suggests fluorescence as a possible cause, but Rayleigh has shown that the "residual blue" observed by Tyndall can be explained by taking into account the second order of small quantities, the residual blue varying inversely as the *eighth* power of the wave length. The occurrence of powers higher than 4 is thus accounted for. Powers lower than 4 can be ascribed to larger particles, and Boutaric shows that from 100,000 particles per cubic centimeter of radius 0.05 micron, to 1,000 of radius 0.1 micron, are sufficient to account for the excess of atmospheric absorption over that which can be ascribed to the air molecules. These numbers are not excessive for the persistent nucleation of the atmosphere. The changes in the polarization of sky-light indicate that the particles causing these changes are relatively large (approaching the wave length of light). Rayleigh's theory has received many other experimental verifications.

Rayleigh's theory assumes that the particles are dielectrics, and so does not apply to the metal sols, which show such beautiful and varied colors. A theory of scattering by small particles was worked out by J. J. Thomson, assuming them to be perfect conductors, and Maxwell-Garnett, Mie, and others have extended the theory to larger particles, and imperfect conductors. A large number of researches on gold and silver sols have shown that the absorption band crosses the visible spectrum toward the red, with increasing size of submicroscopic particle, just as the theory demands. The spectral absorption data of Pihlblad are most complete, showing but one absorption band, and confirming quantitatively Mie's theory. As the particles approach molecular dimensions, the absorption spectrum approaches that of the molecular solution. These colors are so characteristic of

the particular molecule, however, that no simple theory can apply. Rayleigh's theory owes its simplicity to the fact that the selective effect of scattering is not specific, all non-conducting particles looking alike when small, just as all coarse dispersions of these substances appear white. Our discussion of turbidity measurements will be limited to dispersions of such colorless transparent substances, the specific absorption of which can be neglected, except as represented by the ordinary index of refraction.

Turbidity measurements

The word *turbid* is derived from the Latin verb *turbare*, to disturb. The same meaning is attached to the French *trouble*, and to the German *trübe*. Hence any medium containing small particles in suspension is turbid. While the original meaning of the word had no direct reference to the eye, turbidity has become associated with the appearance of a turbid medium. The small particles scatter light in all directions, making their presence strikingly evident.

Although turbidity seems to be the word most generally used to express the characteristic optical properties of dispersions, it has been defined usually as a concentration, ignoring the other variables under the assumption that they are constant. Qualitatively the word turbidity is used to express the physical properties of a dispersion which afford optical measurement. For a given dispersion each method of measurement gives a characteristic value, but all such values are intimately related optically. The word turbidity will hereafter be used in a quantitative sense as the name for this class of optical quantities. The value of the turbidity, by any method of measurement, must not depend upon the intensity of the light used to observe it, although it may depend upon the color.

The methods fall into three groups, those which measure: (1) the ratio of the Tyndall intensity to that of the incident light, (2) the diffuse density, or logarithm of the reciprocal of the diffuse transmission of a layer of the dispersion, and (3) the depth at which a target disappears beneath the layer of turbid medium. Instruments which measure the Tyndall ratio are

called tyndallmeters when the sample is measured directly, and nephelometers when the sample is compared with a standard of known concentration. Instruments which measure the transmitted light, either by the photometric or by the extinction criterion, are called turbidimeters. The three types of turbidity measures will be called, respectively, the Tyndall ratio, the density, and the extinction index. Their characteristics will be considered under instruments and standards, but it may be remarked here that the Tyndall ratio is the only turbidity sensitive to extreme dilutions, while the only one available at high concentrations is the extinction index.

Whatever method is used, the turbidity (T) is proportional to the concentration (C) and to the depth (χ) as a first approximation. Its dependence on color is intimately bound up with size of particle. For particles small compared with the wave length of light, Rayleigh's law (22) states that the Tyndall ratio is proportional to the cube of the particle size (d) and to the inverse fourth power of the wave length (λ). But particles larger than a wave length merely reflect the light from their surfaces, and so the turbidity is proportional to their total surface. We may combine both these relations in a single formula for the turbidity

$$T = \frac{kC\chi d^3}{d^4 + \alpha \lambda^4} \quad (26)$$

where k and α are constants.

Formula (26) holds approximately for both very large and very small particles, but in the transition region, which is probably from a centimicron to a micron, it may depart from the facts more widely. It indicates a very sharp maximum in turbidity when $d/\lambda = (3\alpha)^{1/4}$, for a given concentration and depth. The constant k is specific both to the dispersion and to the method of measurement, but the constant α probably depends upon the method only.

As a function of concentration and depth, equation (26) is of the same form as Beer's law for molecular absorption. For equal turbidities dilutions are proportional to depths, and since

the relation is usually tested by diluting a standard dispersion, we shall call it for brevity the *dilution law*. When turbidity is the diffuse density ($T=D$), a comparison with the exact expression (12) shows that the rejectance factor has been neglected, so that (26) holds only for very thin layers, or for very dilute dispersions. For the very concentrated dispersion opal glass, Bloch and Renwick found a power function of the depth to fit their data, the exponent of (χ) having the value of 0.86. The author (4) has used the same approximation formula in his study of the Kober nephelometer, obtaining an exponent 0.904 with a silica suspension containing 120 parts per million, over a four-fold range in depth.

The same formula was also found to fit the variation of the Tyndall ratio with the depth, giving the exponent 0.924. Kober's parabolic approximation formula is equivalent to the power formula for exponents near unity, and his results on ammonia give an exponent 0.9342. Tolman and coworkers (3) have given the calibration curve of their tyndallmeter over a four-hundred fold range in concentration, for silica particles 1 micron in diameter. The ratio of the turbidity to the concentration increases in the range from 3 to 300 mgm. per liter, and then decreases again for higher concentrations. From 6 to 300 mgm. per liter their data fit the power formula, the concentration exponent 1.225 giving an average deviation of 5 per cent. Mecklenburg (4), however, obtained the dilution law with sulfur sols on his tyndallmeter over a thousand fold range in concentration, with an a.d. of less than 0.6 per cent, but this must be fortuitous, for the reproducibility of his instrument is no better than 3 per cent, and some systematic departure from simple proportionality is required by the complicated exact expression (17). Kleinmann claimed that his nephelometer followed the dilution law, but Owe has shown that this is true only for very dilute dispersions. For $m/200$ barium sulfate, the depth increased as the 0.85 power of the depth of his standard sol, whereas any power function between turbidity, concentration and depth, regardless of exponents, should give direct proportionality.

Wilke and Handovsky have made very careful measurements

of the Tyndall ratio for depths varying from 3 to 24 mm., on gelatin and dextrin solutions, with both red and blue color filters. Their results fit the formula

$$T = T_{\infty} [1 - \exp(-\alpha x)] \quad (27)$$

the constant α ranging from 0.12 to 0.21. This expression indicates that the Tyndall ratio approaches a saturation value (T_{∞}) for very thick layers, which agrees with the exact expression (17). It is quite unlikely, however, that the exact formula reduces to such a simple form as (27) except as a rough approximation. I have shown (4) that it does not even approximately apply to Kober's nephelometer because the illumination is not uniform. The calibration curve of the tyndallmeter designed by the author (3) approximates a power function of the concentration with an average deviation of 6.6 per cent over a two-thousand fold range, with a concentration exponent of 0.838. The systematic deviations, however, show that in reality the law is much more complicated. The best fit with formula (27), using C in place of x , gives an a.d. of 19 per cent, so that over this wide range the power function is a much better approximation.

When the turbidity is measured by the extinction criterion the product of concentration and depth is only approximately constant. In my study of the turbidity standard of water analysis (3), the exponent of the vanishing depth of the turbidity rod of 1902 was found to vary all the way from 1.1 to 2. It is therefore obvious that the only feature common to all the instruments for measuring turbidity is a rough approximation to the dilution law over limited ranges. The exact theory shows that the reason for this is inherent in the optical properties of dispersions themselves, and is not due to any "imperfections" in the instruments. To be sure, each instrument has its own shape factors and its own edge effects, but even if these could be eliminated, the dilution law (26) would still be merely an approximation. There is little choice between the three measures of turbidity (1) Tyndall ratio (2) diffuse density and (3) extinction index, as to the range over which they approach the dilution law. For practical purposes, each instrument must be calibrated

empirically over the concentration range of interest, and so the turbidity might as well be *defined* by the dilution law itself. This, however, leads to the whole question of standards, which will be considered later.

Color and size of particle

The experimental determination of the relation between turbidity and size of particle is quite difficult, especially with submicroscopic particles. It has been attempted by Mecklenburg (4), Tolman, Gerke and coworkers (3), Bechhold and Hebeler (2), and by Owe. Mecklenburg found Rayleigh's law to hold for Sven Odén's sulfur sols of graded size, from 5 millimicrons to 0.1 micron, with an average deviation of about 23 per cent, which is very good for such difficult measurements. It must be remembered that the cube of the diameter increases 8000 fold over this range, so that the Tyndall ratio is an extremely sensitive test of Rayleigh's law.

For sulfur particles between 0.1 and 1 micron in diameter, Mecklenburg found the Tyndall ratio to increase less rapidly than Rayleigh's law, indeed approximately as the first power of the diameter, and inversely as the square of the wave length. There is evidence of a discrepancy in his data for this region, however, for the Tyndall intensity at 0.1 micron particle size is almost stationary, and yet increases more rapidly from 0.3 to 0.8 micron. Tolman (2) and his coworkers have obtained a large amount of data on smokes which indicate that the Tyndall ratio actually decreases as the particles grow in size even with submicroscopic particles 0.1 micron in diameter. Of course the laws may be quite different for dispersions in air, but their results with silica suspensions in water steadily increase with increasing fineness down to particles 1 micron in diameter, so that particles which show a maximum Tyndall intensity must be below 1 micron in size. Bechhold and Hebeler found the maximum to occur at about 0.8 micron with barium sulfate dispersions in glycerol, and Owe, working later in the same laboratory, found the maximum around 0.2 micron, with the same

dispersions. It is well known in the paint industry that the covering power of white pigments increases with the fineness right down to 0.1 micron, as Green has shown. With photographic emulsions, also, the turbidity increases with fineness of grain right down to the limit of resolution of the microscope, which is 0.2 micron. In spite of the great care of Mecklenburg's work, therefore, we must question his data for particles approaching a wavelength in size.

There is one difference between Mecklenburg's data, and that of the others, that may account for the discrepancy. In his commendable effort to eliminate the effects of secondary scattering, he extrapolates his values, with high concentrations quite considerably, in spite of the fact that the data themselves show maxima. With the smaller intensities the extrapolations intersect the axis normally, and so his data for the smaller particles are unambiguous. The results of Bechhold and Hebler on barium sulfate sols with particles ranging in size from 4 to 50 millimicrons are directly proportional to the diameter with an average deviation of 1.6 per cent. It is hard to reconcile such data with those of Mecklenburg, which varied as the cube of the diameter. Certainly such a discrepancy cannot be ascribed to lack of uniformity in particle size, or to inaccurate ultra-microscopic measurements of particle size.

Particles above a micron in size can be measured in the microscope without difficulty, and here there is no disagreement between observers. Tolman and his coworkers find the Tyndall intensity proportional to the surface of silica particles above 2 microns in diameter with an average deviation of 5 per cent, and Bechhold and Hebler agree with an a.d. of 11 per cent for barium sulfate-glycerol sols. Owe has found the Tyndall intensity to decrease very slowly as the sulfate particles increase in size from 0.2 to 0.6 micron, eight different dispersions in glycerol and in glycerol-alcohol giving intensities inversely proportional to the 0.58 power of the diameter with an a.d. of 4 per cent.

Pihlblad has measured the density of sulfur sols in the spectrophotometer over a very wide range of wavelengths and particle size. He confirms Rayleigh's law as regards wavelength with an

a.d. of 8 per cent, but the relation of density to particle size is not very clear for particles below 5 centimicrons. For particles between 5 and 15 $c\mu$ the ratio of density to diameter is constant to 20 per cent, while above 15 $c\mu$ the surface law is definitely followed. The turbidity maximum is therefore between 0.1 and 0.2 μ , which agrees with Owe's results with barium sulfate sols, but disagrees with those of Mecklenburg on sulfur sols. Moreover, Pihlblad has found the product of density and diameter constant for every type of sulfur sol he prepared. Thus by Sven Odén's method of fractional coagulation with sodium chloride, the average deviation was 5 per cent for particles between 16 and 55 $c\mu$, with alcoholic sulfur solutions precipitated in water the a.d. was 29 per cent between 15 and 22 $c\mu$, and with sulfur sols obtained by grinding the a.d. was 15 per cent between 12 and 22 $c\mu$. Even with gold sols, which show strong selective absorption, the surface law was followed at wavelength 436 $m\mu$ with an a.d. of 8 per cent for particles above 0.1 micron in diameter. These results indicate that the turbidity maximum occurs at particle sizes between 0.1 and 0.2 micron.

On the other hand, Owe has obtained with a wedge colorimeter densities which increased in direct proportion to the diameter right up to 1 micron with the very same barium sulfate dispersions which gave maximum Tyndall ratios at 0.2 micron. Here is another major discrepancy which must be cleared up. It may be connected with the shape of the particle, as Owe suggests. His results indicate that the Tyndall ratio is more sensitive to the dispersing medium than is the diffuse density, and that the shape of the particles also varies in different dispersing media. It must not be forgotten, however, that as the Tyndall ratio increases, its relation to the concentration and the depth becomes extremely complicated, as shown by equation (17). Within its range the turbidimeter is to be preferred over the nephelometer, because the exact theory of the density of concentrated dispersions is so much simpler, and gives a closer approximation to the dilution law. Nevertheless, Owe's density results leave the question of where the turbidity maximum occurs still in doubt. The interpretation of density measurements will be considered later in the discussion of turbidimeters.

There is quite a different method of obtaining information about size of particle, which is independent of the color, namely, the degree of polarization of the Tyndall beam. Boutaric has obtained some quantitative data on silver chloride dispersions. They indicate that the depolarization ($1-P$) of the Tyndall beam is proportional to the density (transmitted light), with an average deviation ranging from 5 to 12 per cent for the different growth curves. The ratio of the density in blue light to that in red is constant to 3 per cent, so that the density is inversely proportional to the 3.1 power of the wavelength, which approximates Rayleigh's law. With alcoholic mastic dispersions in water, however, the growth of the particles was practically completed before any measurements could be taken.

It would seem that the turbidity growth curve itself should yield some information regarding the size of particle, if any of the laws of particle growth, above outlined, were followed. For if the diffuse density is a power function of the diameter,

$$D = kd^p \quad (28)$$

and it is necessary merely to substitute the p 'th root of the observed density in place of the diameter (d) in the law of growth to see if it represents the facts. When this is done with Boutaric's data on silver chloride dispersions, however, it is found that none of the simple laws of growth, the diffusion, surface, or coalescence laws, yield high enough values of the exponent to be reasonable, as shown by the drift in the constants in table 3. In other words, the actual growth of the particle is much slower, than would occur on a pure surface of the dispersed phase. This indicates that the limiting factor in particle growth is the poisoning of the surface by adsorbed layers. Since the precipitation of silver chloride is comparatively rapid, the dominance of adsorption phenomena over particle growth must be of fairly general occurrence in dispersions, and this is indicated by their frequent persistence in the dispersed condition for such extensive periods of time. Turbidity growth curves, therefore, which are most easily obtained, present an interesting problem for interpretation, and will probably yield considerable knowledge of adsorption

phenomena. Incidentally, this dominance of adsorption emphasizes with what meticulous care the technique must be standardized if reproducibility is to be expected in turbidity measurements.

TABLE 3
Particle size from turbidity growth curves
(Boutaric's data on AgCl dispersions 6 mgm./l.)

t (min.)	D/D_m	$p = 6$	$p = 4.5$	$p = 3$	$p = 2$
<i>Application to diffusion law of growth</i>					
Relative values of $\log(1 - x^3)/t$					
2	0.278	1627	120	708	344
5	0.405	879	68	451	259
10	0.572	613	51	369	250
30	0.855	374	34	279	226
60	0.990	384	35	167	304
Average.....					277+13%

$p = 6$	$p = 3$	$p = 1$
<i>Surface law of growth</i>		
Relative values of $F(x)/t$		
292	165	835
132	82	496
77	52	362
36	27	217
(24)	(22)	201

t (min.)	D	$p = 6$	$p = 3$	$p = 1$
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<i>Coalescence law of growth</i>				
Relative values of $(x - x_1)/(t - t_1)$				
2	0.111			
5	0.162	173	291	170
10	0.229	129	222	148
30	0.342	59	106	82
60	0.396	33	59	49

The laws of particle growth presented above are adapted to testing the relation of turbidity to size of particle because the dispersions have been assumed to be uniform, that is, all the

particles have been assumed to be exactly alike. Of course, this is hardly ever the case, particularly during growth. In Smoluchowski's theory of coagulation the particles are taken to be all of the same size at the start, but after coalescence the aggregates are distributed in size because of the varying probabilities of collision. Taking ν_1 original particles, after the lapse of time t , ν_1 single particles are left, the others having coalesced into aggregates, ν_2 of two, ν_3 of three, ν_4 of four particles, etc. Smoluchowski then shows that

$$\nu_1 = \nu_0/(1 + \alpha)^2, \nu_2 = \nu_1 \chi, \nu_3 = \nu_2 \chi, \text{ etc.} \quad (29)$$

where $\alpha = 4R\pi\epsilon D\nu_1 t$, and $\chi = \alpha/(1 + \alpha)$. Every time a particle diffuses into the sphere of action of another, the constant probability (ϵ) of coalescence is assumed. R is the radius of this sphere, and D is the diffusion coefficient of the original particles. Westgren and Reitstötter have confirmed the theory on gold sols, finding R to be not much greater than the diameter d_1 of the single particles (see Svedberg (2) p. 218). It is clear that the sphere of action cannot long remain of constant radius, as the aggregates continue to increase in size, but must itself increase. Moreover, the persistence of coarse dispersions for long periods of time shows that the probability ϵ must decrease, probably due to surface poisoning by adsorbed layers, for there is no other factor in Smoluchowski's theory to arrest the growth of the particles.

The distribution of aggregates as a function of the time, indicated by (29), determines the curve of turbidity growth when the relation between turbidity and size of particle is fixed. If the aggregates are all assumed to be spherical, we have $d_2^3 = 2d_1^3$, $d_3^3 = 3d_1^3$, etc., where d_1 is the diameter of the single particle, d_2 that of the double, d_3 that of the triple particles, etc. Assuming Rayleigh's law, the partial turbidities are $T_1 = k\nu_1 d_1^6$, $T_2 = k\nu_2 d_2^6$, etc., and their sum, the total turbidity (T) is therefore, from (29)

$$T = k \nu_1 d_1^6 [1 + 4\chi + 9\chi^2 + 16\chi^3 + 25\chi^4 + \dots] \quad (30)$$

But the series in χ is simply $(1+\chi)/(1-\chi)^3 = (1+2\alpha)(1+\alpha)^2$, and since $(1+\alpha)^2$ cancels from ν_1 , (30) reduces to

$$T = T_0 (1 + \beta t) \quad (31)$$

where $T_0 = k\nu_0 d_0^6$ and $\beta = 8\pi\epsilon RD\nu_0$. The turbidity of the dispersion, therefore, increases linearly with the time as long as the particles remain small enough for Rayleigh's law to hold. During the transition to large particles, which diffusely reflect the light in proportion to their total surface, there is a period when the turbidity is proportional to the volume of the particles, and during this period the total turbidity remains constant, for the series $[1 + 2\chi + 3\chi^2 + 4\chi^3 + \dots] = (1 + \alpha)^2$. Thereafter, the turbidity falls, although the series is too complicated to evaluate because the surface law gives coefficients to the $2/3$ power. The application of Smoluchowski's theory, therefore, seems to explain the general shape of the turbidity growth curves qualitatively, at least, without recourse to any surface poisoning by adsorbed layers. Unfortunately it is not adapted to a quantitative test, but it would probably be found that relatively stable dispersions grow more slowly in size of particle than Smoluchowski's theory would indicate, because of surface poisoning by adsorbed layers. Nevertheless, even when the particles remain uncontaminated the turbidity must increase to a maximum and then gradually decrease as the particles grow in size.

III. INSTRUMENTS AND STANDARDS

The oldest method of measuring turbidity was by means of extinction criterion. For over a hundred years the oceanographer has lowered circular discs of canvas into the sea to measure the depth at which they disappeared. This method is an obvious outgrowth of the simple observation of objects beneath the surface of the sea, which in clear tropical waters are sometimes visible to a depth of over 80 fathoms. The platinum-wire method, now standard in water analysis, originated when Hazen stuck a pin into a piece of wood and lowered it into sewage until it vanished. The earliest form of laboratory apparatus using

the extinction criterion was the diaphanometer designed by Horning in 1876.

Kober (4) refers to Mulder (1859) as the first to use turbidity in the determination of atomic weights. Stas estimated the turbidity of his filtrates by pouring them into large flat-bottomed tubes, and observing an illumined scale below holes over which the tubes were placed. Richards did not consider this method delicate enough, and so designed his nephelometer in 1894, the first instrument to exploit the extraordinary sensitiveness of the Tyndall phenomenon. Nevertheless, the disappearance principle continued the favorite in water work, probably because of its simplicity and freedom from difficulties due to color and to comparison standards. Extinction turbidimeters were designed by Parmelee and Ellms, Jackson, Leighton, Weaver, Smith, and others.

One advantage of the disappearance method is its great range. In field work the United States Geological Survey standard platinum wire can be used in streams of crystal purity, or in rivers of solid mud. Indeed, the extinction criterion has found great usefulness in measuring the covering power of paint pigments, and the fineness of grain of photographic emulsions, dispersions so concentrated that even the diffuse density does not vary appreciably with the depth. Sheppard and Elliot have described a dispersimeter in use at the Eastman Kodak Laboratory, and Pfund a cryptometer for grading the covering power of paint. Renwick has used the extinction criterion combined with a turbid wedge at the Ilford Laboratory in England for many years, and Renwick and the author have developed several forms of the wedge turbidimeter for use at the Redpath Laboratory of the Du Pont Company, but no descriptions of these instruments were published. The possibilities of the extinction criterion for concentrated dispersions are demonstrated by the recent microturbidimeter of Conklin, which in his hands rivals the photometric criterion in precision.

The nephelometer of Richards was further improved by Richards and R. C. Wells in 1904, and placed upon the market by the International Instrument Company of Cambridge, Mass.

Richards always insisted on limiting the nephelometer to the comparison of an unknown with controls of exactly the same age and history, and was quite shocked when Wells later used ground glasses of graded densities as constant standards. The warning of Richards (3) sounds prophetic now in view of the neglect of the question of reproducibility on the part of many workers. Nevertheless, Wells took a step in the right direction, for his constant standards enabled him to study reproducibility and the growth of turbidity. The causes of the differences between turbidity and concentration in sulfate determinations were studied by Muer, who pointed out the importance of fixing the acidity and dilution within certain limits before precipitation.

The wide application of the nephelometer in biochemistry was early recognized by Kober (1913). He developed the instrument and made it available through Klett (New York City), his final design representing a considerable improvement over the model of Richards and Wells. Impressed by its extreme sensitiveness, with more industry than caution he and his associates announced methods for quantitating proteins, casein, proteases, nucleases, phosphorus, etc. He seemed to think the trouble with previous work was in the instrument, and so did not stress sufficiently the difficulties of reproducing precipitates, which accounts for the prejudice against the nephelometer among critical workers. Claims of an accuracy better than 1 per cent, made by Kober, Holker, Kleinmann, Weinberg, and others for their instruments are quite extravagant. The limiting factor in turbidity measurements is the reproducibility of the dispersion, not the precision of the instrument. Even crude instruments are sufficiently accurate to show that such claims are quite beside the point.

Meanwhile Bloor (1914) had converted a Dubosecq colorimeter into a nephelometer and applied the method to fats in milk, blood, etc. His use of a single standard triolein emulsion was criticised by Csonka (1918). The work of Bloor and Kober aroused considerable interest among American biochemists, as shown in the appearance of rapid methods for acetone by Marriott, for proteins in urine by Folin and Denis, for ammonia

by Graves, and for calcium by Lyman. Nephelometric methods were also developed by Woodman, Gookin and Heath for the essential oils, and for mustard gas by Yablick, Perrott and Furman.

After the war the literature began to show the interest in nephelometry among the biochemists in Europe. In Germany the Kleinmann micro-nephelometer, produced by Schmidt and Haensch, represents an optical refinement of the original model of Richards and Wells. Leitz, and H. Krüss of Hamburg also manufacture micromodels of the Duboscq, which can be used for measuring both the density and the Tyndall ratio. Pellin at Paris produce the miniature Duboscq of Baudouin and Bénard. Chénéveau and Audubert have also described a nephelometer. In this country, Bausch and Lomb, and Spencer produce micromodels of the Duboscq, so that there is now available a choice of instruments provided with excellent photometric fields. Many similar instruments have also been described in the literature, usually attempts to use apparatus at hand, or to save expense.

Some biochemists have preferred to use the Duboscq as a turbidimeter matching the densities by transmitted light. During the war in France, Vlès, de Watteville and Lambert developed an "opacimètre" for counting bacteria in flasks which could be sterilized. Color filters and a neutral wedge were used to measure the densities. The nephelometer of Weinberg in Germany we would call a turbidimeter, for the density of a variable depth is matched in a Lummer-Brodhun cube against a standard field controlled by nicol prisms. The extinction criterion has been perfected at the Prudential Laboratory, and the "scopometer" developed by Exton extends its range to high dilutions. In its present form the scopometer is unique in applying both extinction and photometric criteria to both color and turbidity measurements. The extinction criterion was also applied to biochemical work in England by Holker.

The first tyndallmeter was that of Mecklenburg and Valentiner (1), made by Schmidt and Haensch, but it has been considered a research instrument too complicated for general use. The

tyndallmeter of Tolman and Vliet was designed especially for smokes, and has been used by Drinker in his studies of air pollution. The author's (3) tyndallmeter was designed frankly for research upon the optics of dispersions.

Densitometers, which are used to measure the density of photographic images, are of course turbidimeters using the photometric criterion, but the rejectances of the black silver deposits are so small that the photographic density follows the dilution law to a high degree of approximation. Bloch and Renwick used a densitometer to obtain the densities of opal glass given in table 2. The decreasing first differences show that the densities soon become insensitive to depth. Indeed, the exact expression (13) shows that the rate of increase of the density with the depth begins at $\alpha(2P-1)$ for $\chi=0$ and falls to α for great depths. When P is not much greater than unity, therefore, the density follows the dilution law fairly closely.

Nephelometers and tyndallmeters

One may well pause before such a profusion of instruments and ask what qualities are desirable in a nephelometer or in a tyndallmeter. Most photometrists will agree that a prime requisite in both types is a dividing line which vanishes completely when the fields are matched. A black or a bright dividing line between the fields hurts the precision of the match, and multiplies the effort and fatigue of the eye. The mechanism for controlling the brightness should move quickly and easily, for slowness leads to indecision, but ratchets and pinions must not have "backlash," or "creep" between the match and the reading of the scale. The scale should be convenient and illuminated without glare.

The source of light should be scrutinized more carefully than is usual in colorimetry. It is best to have some sort of standard available to check up the constancy of the illumination frequently. Any lack of rigidity in the lamp supports, or possibility of movement, may become a source of serious error. The worst trouble, however, is the color difference between the comparison fields. In the nephelometer, when standard and unknown are

identical except in concentration, a perfect color match should be possible, so that any color difference indicates a color or brightness difference in the incident illumination, just as it does in Duboscq colorimetry. More careful adjustment at the start should eliminate this difficulty. Tyndallmeters should be provided with color filters to overcome the color difference between the constant standard and the sample, and monochromatic filters are advisable on the nephelometer as well, because the Tyndall ratio is a function of the color.

Bechhold and Hebler (1) have reported an interesting phenomenon with two colored hydrosols, using the Kleinmann nephelometer. While they check the dilution law with barium sulfate sols with an average deviation of 0.07 per cent, red kollargol, and blue indigo sols give departures over 100 per cent in some cases, even with color filters over the eyepiece. But when they employ these same solutions, or gelatin filters dyed by them, between the source and the instrument, so that the incident illumination is properly filtered, the departures from the dilution law are again reduced to less than 1 per cent. From this it would appear that these sols are decidedly fluorescent, for otherwise it would make no difference where the color filter is placed.

In choosing color filters to limit the spectral region under observation, high transmission of the dominant hues must be combined with a very sharp edge to the absorption band. The effective wave length is usually near this edge, not at the transmission maximum of the filter, because of the adsorption of the sample, or else due to the drop in visibility at the extremes of the spectrum. For example, the Wratten Stage red no. 27, which is quite stable to light, has a density greater than 2 for wave-lengths below 58 centimicrons, and so the resultant dominant hue is in this region when used with a nephelometer or tyndallmeter on dispersions which give a blue Tyndall beam. The Wratten Stage blue filter no. 47A, with density above 2 for wave-lengths above $51\text{c}\mu$, results in this blue-green color when used over a red solution. The Wratten Mercury green monochromatic filter no. 62, or no. 74 (e), which is practically the same, are very good filters for limiting the spectrum to the

regions of maximum visibility. These gelatin filters are not as permanent as colored glasses, which may be used as control standards, but they are much superior both in transmission and in the sharpness of edge of their absorption bands. With reasonable protection from exposure to light, especially ultraviolet, they are quite stable for years, and they can be accurately reproduced.

There is one danger to guard against religiously in both nephelometers and tyndallmeters, namely stray light. Dispersions are like sponges, integrating the illumination from every source. All instruments should be enclosed unless working in a dark room, and the interior should be matt black. Dust and scratches on the optical parts should be avoided much more carefully than in other optical instruments. But it is better in the course of an investigation not to disturb the adjustments than to run the risk of a discontinuity in the readings due to cleaning and readjustment. Instruments should be sturdy, the adjustments should be reduced to a minimum, lamps and other parts that have to be replaced should be so mounted that there can be no question whatever of their reproducibility. The best tests of stray light are the Zero readings, both with the cells empty and with them full of optically clear distilled water.

With scrupulous cleanliness and care in the technique, the Tyndall criterion is capable of extraordinary sensitivity. Indeed, the method has been used to measure the light scattered by gas molecules themselves, first accomplished by R. J. Strutt, the present Lord Rayleigh, and since developed in extensive researches on both gases and liquids by Raman, Martin and others.

The exact theory of the density of concentrated dispersions is so much simpler than that of the Tyndall ratio, and follows the dilution law so much more closely, that nephelometers and tyndallmeters should be used only for extreme dilutions beyond the range of turbidimeters. To show how far the Tyndall ratio departs from the linear relation of the dilution law, figure 2 has been constructed from Wilke and Handovsky's results. The dotted curves through the observed points are fitted by the

approximation formula (27) (solid curve) with an average deviation of 1.9 per cent for the 10 per cent gelatin solution in blue light, an a.d. of 2.5 per cent for the gelatin in red light, and an a.d. of 0.7 per cent for 10 per cent dextrin in blue light. Ruby red and cobalt blue glasses were used as color filters. Results like these make one wonder if the straight lines obtained with such precision on some nephelometers may not be due to some compensating effect. The illumination may increase with the

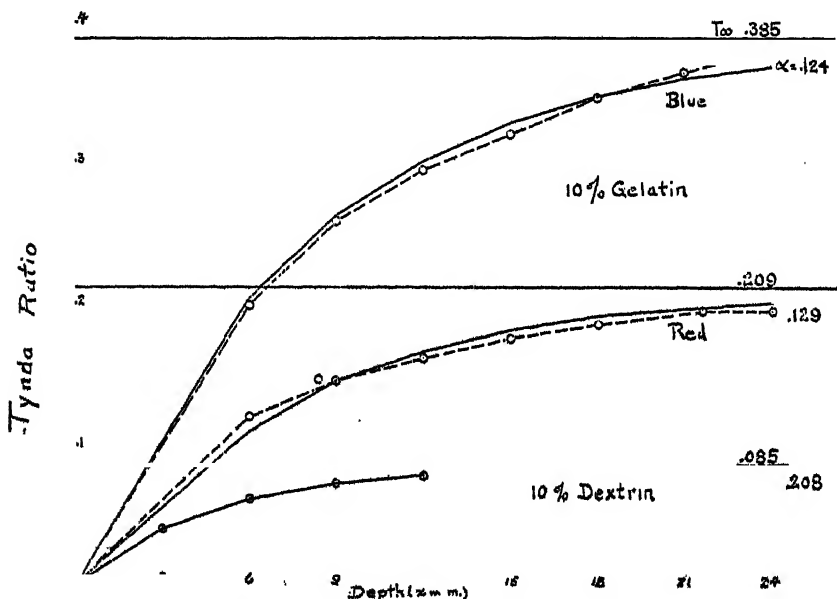


FIG. 2. WILKE AND HANDOVSKY'S DATA

depth, as I have shown occurs on the Kober instrument, or the secondary scattering from layers not directly illuminated may contribute some light not figured in the theory. If this is so, the size of the indirectly illuminated portion must have considerable effect upon the calibration curve. It is obvious, however, that any such empirical compensation must be carefully calibrated before the readings can be interpreted. It is not safe to calibrate with a single stock dispersion, and then assume that thereafter concentrations can be read directly from this curve.

The instrument is really limited to interpolation within a narrow range, just as Richards (3) pointed out in 1906. At extreme dilutions, however, the field for nephelometers and tyndallmeters par excellence, both theory and experiment show that the dilution law is followed.

Turbidimeters

The requirements of a reliable turbidimeter are similar in many respects to those for measuring the absorption of homogeneous media, but there is one very important difference. Since dispersions scatter in every direction light received from every direction, the incident beam must be specified just as definitely for density measurements as for the Tyndall ratio. This is well recognized in photographic densitometry, where the density to parallel light represents more nearly the conditions in practise, while the diffuse density is simpler from the point of view of measurements.

In turbidimetry it is preferable to use the diffuse density, which follows simpler laws and gives more reproducible results. The practical requirement of a small volume of sample, however, introduces edge effects which defy theoretical treatment but which nevertheless, may at least partially compensate for the systematic departure from the simple dilution law which the exact equation (13) requires. Such empirical adjustments, however, always lead to doubt in the interpretation. The dilution law assumes that it makes no difference how closely the particles are packed together, a given number of particles always obstructs the light by the same amount. But in a narrow cell the edge effects upset any such simple relation. If the particles were all crowded into a thin enough layer, the light escaping from the edge could be neglected, while if they were diluted to an extreme, practically all the light would escape edgewise. This shows that the width of the cell is a factor as well as the total number of particles, which contradicts the dilution law. The reason Beer's law holds for molecular absorption is because the molecular scattering is reduced to an utterly negligible quantity by destructive interference, so that all the light is either transmitted regularly, or absorbed as heat.

The extinction criterion possesses the advantage over the photometric criterion of apparent freedom from color difficulties. Anyone can watch an object disappear, whatever its color and regardless of the color of the surrounding field. In practise, therefore, the criterion is easy, except for a feeling of hesitancy which is more pronounced than that with the photometric criterion. In theory the extinction criterion is a measure of the least perceptible increment, or contrast limen (l) of the particular portion of the retina that receives the image of the target, when the adaptation of the eye is fixed by the brightness of the surrounding field (I_x). If I'_x is the target brightness at extinction, and I_o and I'_o are the corresponding brightnesses at the same depth (x) with distilled water in the cell, we have

$$I'_o = I_o (1 + k) \text{ and } I'_x = I_x (1 + l) \quad (29)$$

where k is the contrast factor with distilled water. The regular transmission (t'_x) and the diffuse transmission (t_x) are respectively, assuming the dilution law,

$$t'_x = \exp(-a'Cx) \text{ and } t_x = \exp(-aCx) \quad (30)$$

where C is the concentration of the dispersion, a' and a are constants. The target brightness includes not only the transmitted rays which combine to produce the image, but also a diffuse portion representing secondary scattering. Assuming this to increase with the depth in proportion to $(1 - t_x)I_o$, we have

$$I'_x = t'_o I'_o + b(1 - t_x) I_o \quad (31)$$

where b is a constant. Since $I_x = t_x I_o$, (29), (30), and (31)

$$Cx = \frac{1}{a' - a} \ln \left[\frac{1 + k}{1 + l + b(1 - 1/t_x)} \right] \quad (32)$$

The right hand member of this equation is constant for a given instrument and dispersion except for the small term in b , which can be neglected, and the contrast limen (l) which is personal to the observer. The extinction criterion therefore follows the

dilution law to about the same extent as does the diffuse density, represented by the constant a , provided the visual acuity of the observer remains constant. Any variation in his acuity will change his reading, but not in the same proportion, because l enters in the logarithm. Moreover, different observers will agree in their readings only to the extent that their acuities are the same. Since the visual acuity of each observer is a slightly different function of the color and the brightness level, the extinction criterion is subject to systematic errors which do not vitiate the photometric criterion. Indeed, the photometric criterion is a null method, using the eye as a galvanometer is used in measurements with the potentiometer, while the extinction criterion uses the eye as a direct reading instrument such as a voltmeter. It is a marvel that the eye can be used in such a manner at all, and yet experience shows that normal observers actually do agree to a surprising extent after a little practice together. The personal equation can be largely eliminated by referring the readings to those on a constant standard of about the same value. If the two extinctions appear the same to the observer, the only residual error is the change in his criterion between the readings on the unknown and on the standard.

Equation (32) shows that the extinction index increases slowly when more contrast (k) is used between the target and the field, and decreases slowly when the least perceptible increment (l) grows larger as the result of visual fatigue, etc. In the experience of the author, visual acuity is variable from moment to moment, so that the apparent precision of the extinction criterion is not a safe measure of the real accuracy. Whenever the color difference in the photometric field is eliminated, the photometric criterion is more reliable. For rapid routine work, however, where an average deviation of 5 per cent is permissible, it is much simpler to use the extinction method. With care the average deviation of the method can be kept below 2 per cent, even in routine work, as will be shown later. Incidentally, many authors are quite careless in stating the precision of their measurements. The simplest measure of variability is the average deviation of a single determination, which is used throughout this review.

Turbidity standards

It is not at all certain that it is possible to reproduce a dispersion from specifications with sufficient accuracy for the purposes of a standard. The most that can be expected at present is a fair degree of permanence in a standard, and this rules out dispersions in liquids. Opal glass, however, cannot be obtained of the proper range of turbidities for tyndallmeters, although it is quite satisfactory for turbidimeters. Since it cannot be made accurately to specification, the unit of turbidity should not be defined by opal glass, but it is useful in calibrating instruments. A number of samples of opal glass from different melts would probably be sufficiently permanent to maintain a constant turbidity standard for many years to come, just as incandescent lamps are used to maintain the standard candle.

The specific unit of turbidity might be defined by an arbitrary number, which need not represent the turbidity reading obtained in any actual case. But it is preferable to have the turbidity equal the concentration of the most turbid substance that can be obtained. One could then visualize, from the value of the turbidity, the inherent capacity of the dispersion to scatter and obstruct light. The most that could be expected from a given concentration of material would be a total turbidity equal to 100 per cent of the concentration. Of course, the thickness of the sample to which the specific turbidity refers should be the centimeter. By using the specific turbidity maximum as a unit, the size of particle will be so large that the specificity to color will be small; but to eliminate all ambiguity it should be referred to monochromatic light. The best wave length would be the mercury green line at 546 m μ , which is the most brilliant monochromatic source available, and is very near the maximum of visibility.

The actual evaluation of the standard should be performed by the Bureau of Standards. It must be postponed until more is known about what dispersions give the greatest specific turbidity, but in the meantime the silica standard of water analysis might be assumed to have unit specific turbidity, when measured

at a concentration of a gram per liter. The trouble with the silica standard is that the fineness is specified by the vanishing depth (100 mm.) of a platinum wire 1 millimeter in diameter, "in the open air, but not in sunlight, and in a vessel so large that the sides do not shut out the light so as to influence the results. The turbidity of such water is arbitrarily fixed at 100 parts per million." This is very inconvenient for the laboratory. The platinum wire method is not suitable for a standard, because of the uncertain personal equation of the observer, so that even the water analysts themselves have shown a tendency to avoid its use.

Bechhold and Hebler have suggested as a standard a millimolal dispersion of barium sulfate in glycerol, giving particles 2.5 microns in diameter. It is precipitated by mixing 2-millimolal hydroxylamine sulfate with an equal volume of a like concentration of barium chloride, each dissolved in glycerol. They call this the Kraus standard, after their colleague who developed it. Kraus found the standard to follow the dilution law on the Kleinmann nephelometer from 0.02 to 0.8 millimol with an a.d. of 0.07 per cent. The best results were obtained with equivalent amounts of the sulfate and $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, for which the turbidity was independent of the method of mixing. Heating to 100° changed the turbidity 5 per cent, but no change occurred in an ice bath. For 24 hours the turbidity remained constant within 4 per cent; in eight days it fell 6 per cent. They also prepared a 5 millimolal standard barium sulfate sol with magnesium sulfate. The dispersing medium was glycerol containing 15 per cent isobutyl alcohol. The particles were less than 90 millimicrons in diameter, and the specific turbidity was 60 per cent of the standard. This sol remained unchanged for 6 months. They check von Weimarn's precipitation laws in general, but the isobutyl alcohol complicates the relations because of its slight solubility in glycerol. The dispersity increased with the excess of the barium ion, but contrary to von Weimarn's laws an excess of sulfate ion did not give stable sols. They do not give any data to show the reproducibility of their standard from specification with glycerol, sulfate and barium from different sources, the situation that would have to be met in practice.

The diffuse density is the most reproducible measure of the turbidity when the proper concentration and depth are chosen. But a dispersion which shows the maximum specific turbidity by density measurements is not at all the one which shows a maximum Tyndall ratio, because absorption of light by particles contributes to the density but subtracts from the Tyndall intensity. The tyndallmeter, therefore, must be used to discover the dispersion of maximum specific turbidity, in spite of its limitation to high dilutions. In some respects this is an advantage, for von Weimarn's laws indicate that large particles precipitate only at low concentrations, and one would expect less alteration in the particles on dilution when they are already widely separated. Once the standard dispersion is specified, however, the advantages of both turbidimeters and tyndallmeters could be utilized, for it seems reasonable to hope that a concentration and suitable depths could be found for precise measurements of all three of the measures Tyndall ratio, density, and extinction index.

The calibration of the instruments can be made by assuming the dilution law for a variety of dispersions. Inconsistencies can probably be ascribed in most cases to changes in the particles on dilution. The dilution must be made with the same solution as the dispersing medium, for otherwise the solubility product requires a change in the total mass of the dispersed phase.

Reproducibility of dispersions

No matter how perfect the instrument is for measuring turbidity, until the dispersions themselves can be reproduced its usefulness is limited. It must not be taken for granted that the same technique will always give the same turbidity with the same concentration of the dispersed phase. It is surprising how little data on reproducibility the literature has to show. Probably more work has been published on silver chloride in water than on any other dispersion, and yet Kleinmann concludes after extensive studies that they are not adapted for nephelometry. He says (1, p. 143): "Doch zeigte es sich, dass bei Innehaltung der sich als relativ günstigst herausstellenden Ar-

beitsbedingungen eine überwiegende Mehrheit alle Versuche ca. 66 $\frac{2}{3}$ % gleiche Teilchengrösse ergab und die Proportionalität der Beziehungen bestätigte. Ein Drittel aller Versuche waren vollständig verschiedene Ausfälle. Es müssen bei der Herstellung kolloider Lösungen Einflüsse ein Rolle spielen, die wir entweder nicht kennen oder noch nicht beherrschen." He prefers his phosphomolybdate strychnin compound, which seems to

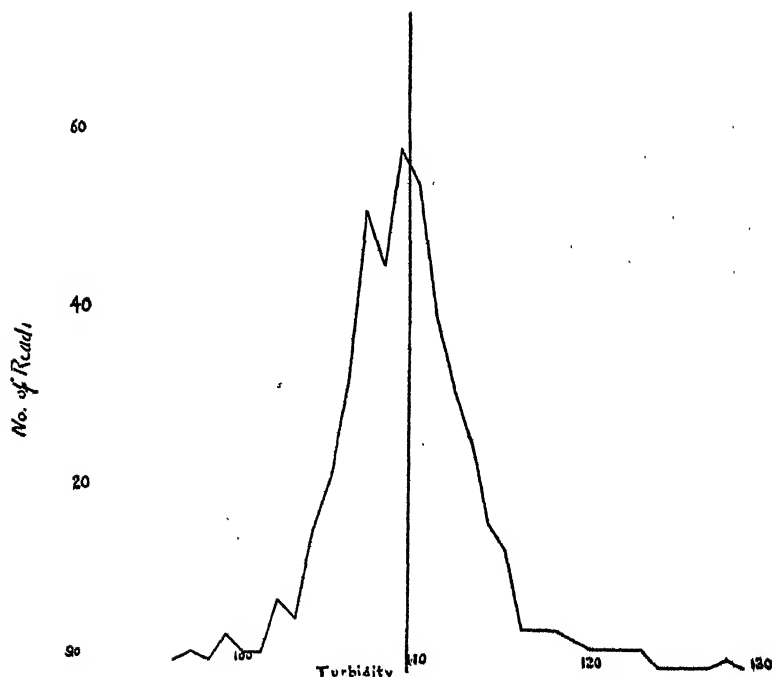


FIG. 3. REPRODUCIBILITY OF TURBIDITY

follow more regular laws. According to Kober, it was unfortunate for the development of nephelometry that silver chloride dispersions were studied first.

In view of the vagaries of silver chloride suspensions complained of by the nephelometrists, it is interesting to see what reproducibility is obtained in the turbidity of photographic emulsions. Figure 3 shows the frequency curve of 442 turbidities, all the

batches of a fixed type measured over a period of twenty months at the Redpath Laboratory of the Du Pont-Pathe Film Manufacturing Company, using the micro-turbidimeter designed by Conklin.² The frequency distribution closely approximates the normal probability law, with a standard deviation of 3.9, or a probable error of 2.4 per cent. The average deviation is 2.6 per cent, and the entire range is 27 per cent. One-half the batches, therefore, fall within a range of less than 5 per cent, one in ten would be expected to deviate by more than 5.9 per cent, and one in a hundred by more than 9.3 per cent. When it is considered that these deviations include all the vagaries of plant production as well as the errors of measurement by at least four observers using the extinction criterion, the data seem sufficient to demonstrate the possibility of reproducing silver halide dispersions. It may be remarked that Kleinmann used an excess of silver, while Boutaric used an excess of chloride. Boutaric cautions against pouring the chloride into the silver nitrate, and while his curves are averages of several runs, he makes no mention of any failure to reproduce. Lamb, Carleton and Meldrum revised Wells' technique on silver chloride precipitation, but do not present any data which shows the reproducibility of their final technique.

Chénéveau and Boussu call attention to the necessity of studying carefully and systematically each method, but the following statement of Hibbard best describes the situation.

Successful turbidimetry depends not only on a satisfactory instrument, but equally as much on a satisfactory method of producing the precipitate to be measured. This must be such that a given weight of the substance to be measured will always produce a precipitate of the same optical quality, so that it will have the same opacity and give the same reading in the turbidimeter. One who has not tried this will hardly realize the difficulty of the problem. By varying the concentration and manner of mixing the reagents, it is possible to produce a precipitate of barium sulfate having 2 or 3 times the opacity of an-

²The author wishes to express here his thanks to Dr. V. B. Sease, Director of Redpath Laboratory, for placing these data at his disposal.

other produced from the same amount of sulfate. Other precipitates are subject to similar variations.

For turbidity measurements the precipitate should be very fine so as not to settle rapidly; yet it must not be of colloidal dimensions. Colloidal precipitates are not uniform in appearance and are not easily compared in the turbidimeter. Moreover, their suspensions are liable to appear colored by transmitted light. Suspensions of large crystals have relatively low opacity and settle very rapidly; therefore, they are not suitable for turbidimetric estimation.

In order to produce a precipitate of uniform physical character, the following conditions must be controlled within somewhat narrow limits: (1) concentration of the 2 ions which combine to produce the precipitate (2) ratio of concentration in solutions mixed (3) manner of mixing (4) time rate of mixing (5) amount of other salts present (6) temperature.

Hibbard found calcium and sulfate techniques that were quite satisfactory, and a fairly acceptable magnesium technique. It is to be hoped that future work will make available a large variety of methods which will reproduce satisfactorily, and thus take full advantage of these convenient optical instruments.

Applications of turbidity measurements

It is evident from the work already cited, that turbidimetry takes its place beside colorimetry as an extremely sensitive method of volumetric chemical analysis. It is already a standard method in water analysis and has established its usefulness on toxic smokes during the war, and on the pollution of air by dust particles. Many raw commercial products are turbid, and must be filtered. Turbidity is a convenient measure of filtration efficiency. In grading the size of particles of pigment, etc., turbidity is a direct statistical measure much more readily determined than the laborious methods of the microscope and ultramicroscope. Tyndallmeters and turbidimeters are particularly adapted to the study of the kinetics of precipitation, coagulation, and peptization reactions, and transformations in sols and gels. Critical opalescence is a sensitive indicator of the critical state.

In the biochemical analysis of blood, urine, spinal fluid, etc., the method has already shown its usefulness, particularly for the proteins for which no comparable color reactions have been found. The extremely sensitive and specific enzyme and precipitin reactions will doubtless yield information by its application. In the standardization of vaccines, and the counting of bacteria and blood corpuscles, some work has already been done. But its field par excellence will undoubtedly be in chemical pathology, for living structures are so delicate that brute methods must destroy the very phenomena they are intended to portray.

In concluding the author desires to express his appreciation of two masters under whom he has worked, Mr. F. F. Renwick and Dr. W. G. Exton, whose intimate knowledge of the subject has exerted a profound influence upon his studies.

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THE INTERACTION OF ELECTROLYTES WITH NON-ELECTROLYTES

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It seems almost impossible, now that another point of view has been presented, that the electrolytic dissociation theory, coming after the work of Faraday, Kohlrausch and Hittorf on the properties of electrically conducting systems, and basing its quantitative calculations on these properties, could have been developed with complete disregard of the fact that ions differ from other molecules in being electrically charged. Eight years after the classical papers of Arrhenius and Van't Hoff, Van Laar (1895) did suggest that the electrical forces have an effect on the properties of electrolyte solutions.² Twelve years later Sutherland (1907), followed by Bjerrum (1909) claimed that strong electrolytes are completely dissociated. Quantitative calculations were given by Van Laar (1900), Milner (1912) and Ghosh (1917). Ghosh's attempt was voted down almost unanimously, partly because it was wrong in theory. The others were almost completely ignored. This action can be only partially justified by the plea that the earlier works were either vague or complicated. Six years after Ghosh, Debye and Hückel (1923) published a simple and sound treatment which has met with general recognition, and which has in the last three years revolutionized the study of electrolyte solutions.

The Debye picture is essentially that, since the ions are charged particles, the electrostatic forces must be taken into account.

¹ Lecture, expanded in parts, delivered at the Rockefeller Institute for Medical Research, April 23, 1926.

² This brief historical introduction does not pretend to be complete. References are given only to those papers which have a direct bearing on the present work.

Consideration of these forces, using elementary electrical theory, shows that there are two effects of ionic charges on free energies or activities. The first is proportional to the square root of the ion concentration in dilute solutions, but approaches constancy as the concentration increases. It is the result of the interaction of one ion with another. The second is proportional to the first power of the concentration. It is the result of the interaction of an ion with any other particle,—with an uncharged molecule or with another ion. In the case of two ions each takes the parts both of an ion and of a neutral molecule.

The study of the first effect is what is generally known as the Debye or Debye-Hückel theory of electrolytes.³ The second was presented by Debye and McAulay⁴ and extended somewhat by me⁵ for the case of an ion and a non-electrolyte, and by Hückel⁶ for the case of two ions. It has received much less attention than the first, perhaps because the existence of any such effect was denied by the classical theory of electrolytic dissociation and its study is not a part of standard courses in physical chemistry. It seems to me that it may be at least as important, as the first, particularly in biological systems, and it is this second effect which I shall discuss today.

GENERAL SOLUTION THEORY

It will be convenient to outline at this point the thermodynamic and solution theory which is to be used later. Consider a system containing n_1 mols of solvent, n_2 mols of a non-electrolyte solute, and n_3 mols of an electrolyte, each molecule of which dissociates into ν ions of valence z_+ and z_- .⁷ The subscript i indicates any specified component. The composition of the system will be expressed by three different systems: the mol fraction, $x_i = n_i/(n_1 + n_2 + \nu n_3)$; the molality, $m_i = n_i/w_1 n_1$; and the molal

³ Debye and Hückel, *Physik. Z.*, **24**, 185 (1923).

⁴ Debye and McAulay, *ibid.*, **26**, 22 (1925).

⁵ Scatchard, *J. Amer. Chem. Soc.*, **47**, 2098 (1925).

⁶ Hückel, *Physik. Z.*, **26**, 93 (1925).

⁷ The symbols used in this paper, with their definitions are collected on page 402.

concentration, $c_i = n_i/V$. w_1 is one thousandth the molecular weight of the solvent, V is the volume in liters of the system. The ionic strength, μ and a similar quantity, Γ , related to the molal concentration, are defined by the equation:

$$\mu/m_s = \Gamma/c_s = (\nu_+ z_+^2 + \nu_- z_-^2)/2 = \nu z_+ z_-/2.^8$$

Let F be the free energy of the system, F_i the free energy of the same amount of an ideal solution of the same concentration, a_i the activity of the component in question, and F_j the free energy of one mol of the pure component in the liquid state, R the molal gas constant, and T the absolute temperature. Equations 1 and 2 define an ideal solution and activity. We will

$$RT \ln x_j = \partial F_i / \partial n_j + F_j \quad (1)$$

$$RT \ln a_j = \partial F / \partial n_j + K_j \quad (2)$$

$$f_j = a_j/x_j \quad (3)$$

define an activity coefficient, f , by equation 3. Combining equations 1, 2 and 3 gives equation 4.⁹ K_j is an arbitrary con-

$$RT \ln f_j = RT \ln a_j - RT \ln x_j = \partial F / \partial n_j - \partial F_i / \partial n_j + K_j - F_j \quad (4)$$

stant depending on the reference state of unit activity coefficient. If this is the pure component $K_j - F_j$ is zero. Although this condition will not generally be met with in our equations, we will omit K_j and F_j and will define for each equation the state in which the activity coefficient is unity.

Since, at the same temperature, the activity of a component is the same in all solutions in equilibrium with the solid or liquid phase of that component, its gaseous phase at some definite

⁸ The second equality sign follows from the equations $\nu_+ + \nu_- = \nu$ and $\nu_+ z_+ = \nu_- z_-$.

⁹ T , p and the quantity of the other components are held constant during the differentiation, so $\partial F / \partial n_j$ is the μ of Gibbs. If a function Δ_j were defined by the equation $-\Delta_j = \partial F / \partial n_j - RT \ln x_j - F_j$, the equations of this article could be developed a little more simply by adhering more closely to Gibbs. The present treatment is adopted because the concepts of activity and activity coefficient appear less abstract to those not mathematically inclined than the functions μ (Gibbs) and Δ_j .

pressure, or its solution in an immiscible solvent at some definite concentration, the ratio of the solubilities, s' and s'' , in two solutions, expressed as mol fractions, is given by equation 5.

$$RT \ln s'_1/s''_1 = RT \ln f'_1/f''_1 \quad (5)$$

If the process in an electrical cell is the transfer of the electrolyte from one solution to another, the electromotive force of the cell is given by equation 6, in which q is the quantity of electricity passing through the cell while one mol of electrolyte is transferred.

$$E = E' - E'' = \frac{RT}{q} \ln \frac{a''_1}{a'_1} = \frac{RT}{q} \ln \frac{f''_1}{f'_1} + \frac{RT}{-} \ln \frac{x''_1}{x'_1} \quad (6)$$

It is obviously convenient to consider solutions of the same mol fraction and to extrapolate to zero concentration of electrolyte. In such a case the electromotive force will be designated by E_x .

For equilibria involving the distribution of the solvent we will use some equations which are exact only at infinite dilution, but which hold approximately in dilute solutions. The subscript $n + s$ will be used later to designate the system, $n_1 + n_2 + n_s$; n

$$\partial F_I / \partial n_1 = \partial F_n / \partial n_1 + \partial F_s / \partial n_1 \quad (7)$$

$$\partial F / \partial n_1 = - P v_0 = - P v_1 / d_0 \quad (8)$$

$$\partial F / \partial n_1 = - Q_0 \Theta / T_0 \quad (9)$$

designates the system in which n_2/n_1 is unchanged but n_s is zero; and $_s$ designates the system in which n_s/n_1 is unchanged and n_2 is zero. v_0 is the volume of one mol of pure solvent, d_0 its density, T_0 its freezing point, and Q its molal heat of fusion. P is the osmotic pressure of the solution, and Θ its freezing point depression.

ELECTRICAL THEORY

An ion may be represented by a charge of electricity uniformly distributed over the surface of a sphere. We will assume that the radius of this sphere—the equivalent radius of the ion—is a property of the ion itself and independent of the medium. This assumption is probably not strictly accurate because part of the

electrical energy of the ion lies in the surrounding medium, so that the equivalent radius is somewhat larger than any mechanical radius of the ion, and it might well vary with the medium. We will restrict our calculations to electrolyte concentrations so small that the interaction between the ions is negligible, that is n_3 shall be very small relative to $n_1 + n_2$. In our computations we will use the Debye-Hückel theory to extrapolate from electrolyte concentrations beyond these limits.

Granting these assumptions the potential at the surface of an ion is given by Coulomb's law, equation 10, and the work of charging the ion reversibly follows as in equation 11 from the definition of potential.

$$\Phi = \frac{e}{D\bar{b}} \quad (10)$$

$$w_e = \int_0^{ze} \Phi de = \int_0^{ze} \frac{e}{D\bar{b}} = \frac{z^2 e^2}{2D\bar{b}} \quad (11)$$

Φ is the potential at the surface of the ion sphere, e is the charge on the ion, D is the dielectric constant of the medium, \bar{b} is the radius of the ion, z its valence, and e is the charge of the hydrogen ion. w_e is the electrostatic contribution to the free energy of the system. If there is no other cause for deviation from the laws of ideal solutions, the sum for all the ions, W_e , is equal to the difference between the free energy of the solution and that of the corresponding ideal solution. This sum is given by equation 12,

$$W_e = F - F_I = \frac{N e^2 n_1 v z_+ z_-}{2D\bar{b}} \quad (12)$$

in which N is Avogadro's number.¹⁰

That completes the electrical theory. It only remains to differentiate equation 12 and to combine the result with the

¹⁰ The equations of Note 8 are used to obtain equation 12 from the summation of equation 11, and \bar{b} is a mean radius of the two ions of such a nature that $1/\bar{b} = (z_+/b_+ + z_-/b_-)/(z_+ + z_-)$.

appropriate equation from the last section. The results will be given in terms of the activity coefficients.

$$RT \ln f_2 = \frac{\partial F}{\partial n_2} - \frac{\partial F_I}{\partial n_2} = \frac{N\epsilon^2 v z_+ z_-}{2D_0 b} \quad (13)^{11}$$

For the non-electrolytes it is possible to give a general equation only if the dielectric constant is known as a function of n_2/n_1 . If n_2 is small relative to n_1 the dielectric constant may be represented by equation 14, in which D_0 is the dielectric constant of the pure solvent, and β is a constant characteristic of the two

$$\frac{1}{D} = \frac{1}{D_0} \left(1 + \frac{\beta n_2}{w_1 n_1} \right) \quad (14)$$

non-electrolytes. Combination of equations 12 and 14 gives equation 15.

$$W_2 = F - F_I = \frac{N\epsilon^2 n_2 v z_+ z_-}{2D_0 b} \left(1 + \frac{\beta n_2}{w_1 n_1} \right) \quad (15)$$

$$RT \ln f_2 = \frac{\partial F}{\partial n_2} - \frac{\partial F_I}{\partial n_2} = \frac{N\epsilon^2 n_2 v z_+ z_- \beta}{2D_0 b w_1 n_1} = \frac{N\epsilon^2 \beta \mu}{D_0 b} \quad (16)^{12}$$

$$RT \ln f_1 = \frac{\partial F}{\partial n_1} - \frac{\partial F_I}{\partial n_1} = - \frac{N\epsilon^2 n_2 v z_+ z_- \beta n_2}{2D_0 b w_1 n_1^2} = - \frac{N\epsilon^2 w_1 \beta \mu m_2}{D_0 b} \quad (17)^{13}$$

Combining equation 17 with 7, 8 and 9 gives equations 18 and 19.¹³

$$P_{n+2} - P_n - P_s = \frac{N\epsilon^2 d_0 \beta \mu m_2}{D_0 b} \quad (18)$$

$$\Theta_{n+2} - \Theta_n - \Theta_s = \frac{N\epsilon^2 w_1 T_0 \beta \mu m_2}{Q_0 D_0 b} \quad (19)$$

¹¹ Because of the peculiar definition of the corresponding ideal solution f_2 is unity when D is infinite. In practice an arbitrary factor is included to make f_2 unity in some specified system, generally in pure water.

¹² f_2 and f_1 are unity when n_2 is zero.

¹³ Equations 18 and 19, which are derived here on the assumption that there is no deviation from the laws of ideal solutions except that due to the electrostatic forces here discussed, may also be derived with the less sweeping assumption that any other deviation in $\partial F_{n+2}/\partial n_1$ is equal to the sum of the deviations in $\partial F_n/\partial n_1$ and $\partial F_s/\partial n_1$.

With the above equations it is possible to express the changes with changing composition in equilibria involving the distribution of any component of a mixture of two non-electrolytes and an electrolyte, if the electrolyte concentration is very small, in terms of the properties of the pure components and one property, the dielectric constant, of the mixture of non-electrolytes. One property of the electrolyte, the apparent radius, cannot be determined at present independently of some distribution measurement.

The relations of these equilibria have been given many explanations, such as hydration, chemical action between the two solutes, or a change in internal pressure. I wish to emphasize the fact that the Debye theory does not deny the existence of any of these factors but merely claims that there must be in addition this electrical effect. Similarly the Debye-Hückel theory does not claim that all electrolytes are completely ionized, or that any specific electrolyte is. It claims only that when there are ions there is an electrical force between them. When this force is taken into account it explains the properties of many salt solutions so exactly that we must conclude with Professor Debye that in these cases, "there are not enough undissociated molecules to show any signs of life." The rest of my talk will be devoted to showing how completely electrical effects will account for the properties of electrolyte-non-electrolyte solutions.

ACTIVITY OF THE ELECTROLYTE

For the treatment of the activity of the electrolyte the Debye-Hückel equation, given in equations 20 and 21, will be needed to extrapolate to zero electrolyte concentration. D in these equations is the dielectric constant of the solution without electrolyte,

$$-\frac{1}{\nu} \ln f_{\pm} = -\ln f_{\pm}^0 = \frac{N_A^2 z_+ z_-}{2DRT} \frac{\kappa}{1 + \kappa a} - B \Gamma \quad (20)^{14}$$

$$\kappa = \left(\frac{8000 \pi e \Gamma}{DRT} \right)^{1/2} \quad (21)$$

¹⁴ In these equations f_{\pm} and f_{\pm}^0 are unity when $n_{\pm} = 0$ and n_{\pm}/n_1 is the same as in the solution under consideration.

π has its usual significance, f_{\pm} is the mean activity coefficient of the ions, a is the mean diameter of their collision spheres, and B , which is a constant for a given electrolyte and given solvent, is an approximation for a complex function of all the variables except the concentrations. a and B have been determined for various electrolytes in aqueous solution, and for hydrogen chloride also in 50 and in 100 mol per cent ethyl alcohol. For hydrogen chloride a appears to be independent of the solvent. For other electrolytes we will assume that this independence is general, and that the ratio of B 's for two electrolytes is also independent of the solvent. For solvents other than water-alcohol mixtures we will assume that B is a function of the dielectric constant. Since $B\Gamma$ is generally only a small fraction of the total effect, any error in these assumptions is not very important.

The constants for aqueous sodium chloride at 25° are $D = 78.8$, $a = 2.35 \times 10^{-8}$, $B = 0.089$; x_{\pm} is 0.0924 and Γ is 5.422 for the saturated solution. From equation 20 we determine that $f_{\pm} = 1.155$, and $a_{\pm} = f_{\pm}x_{\pm} = 0.1067$. With the assumption that $E = 0$ for a solution of unit activity in water, $E = -0.1183 \log a_{\pm}$ for any other solution. Therefore for the saturated solution $E = 0.1150$. This value is the same for the saturated solution in any solvent.

For sodium chloride in anhydrous ethyl alcohol at 25°, $D = 25.2$, $B = 0.0288$; in the saturated solution $x_{\pm} = 0.000713$, and $\Gamma = 0.0122$. By equation 20, $f_{\pm} = 0.540$ and $a_{\pm} = 0.000385$. $E_x = E + 0.1183 \log a_{\pm} = 0.1150 - 0.4039 = -0.2884$. This value is represented by the circle cutting the right margin of Figure 1. The other circles in this figure are determined by similar computations from the solubility measurements in mixed solvents. They do not depend in any way on the theory discussed earlier in this paper.

Applying equations 6 and 13 to E_x for pure alcohol leads to the result, $b = 1.31 \times 10^{-8}$ cm. for sodium chloride. This is not very different from the mean ionic radius found for the solid salt. With this value of b and the dielectric constants of water-alcohol mixtures, the same equations give the curve of figure 1, which is thus independent of the solubility measurements in mixed sol-

vents. Yet it corresponds very closely to the best smooth curve which could be drawn through the circles. It is nearly a straight line, but the deviations from a straight line would be considerably larger.

Let us consider what might prevent the simple theory from describing the facts so accurately. We have said that b might not be independent of the solvent. In computing the mol fraction it has been assumed that the molecular weight of water is eighteen and that of alcohol is forty-six; if there were any polymerization

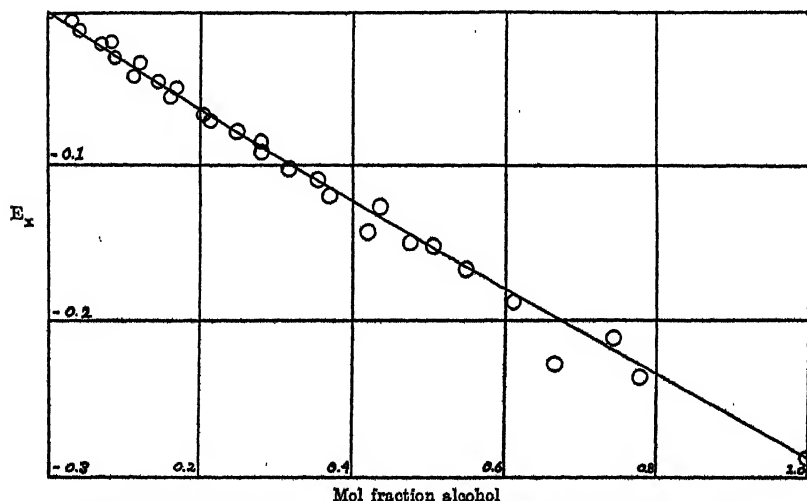


FIG. 1. SODIUM CHLORIDE IN WATER-ETHYL ALCOHOL MIXTURES

of either species or any compound formation between them this assumption would be inaccurate. If either of the ions reacted with either of the non-electrolytes the shape of the curve would be altered. It is also assumed that, except for the electrical effects, the constant of Henry's law is the same in each solution. No non-electrolyte—even among the noble gases where we should least expect specific actions—behaves so simply. In spite of all this the agreement is as shown. This figure explains better than anything else I know the request Professor Debye made to me when I was preparing this material for publication, "Be sure to tell them that the theory works better than it has any right."

To illustrate the method of the inverse problem we will compute the solubility of sodium chloride in 50 mol per cent (71.9 weight per cent) alcohol, for which $D = 37.1$ and $B = 0.161$. From equations 6 and 13, $E_x = -0.1521$. So $E - E_x = 0.2671$, and $a_{\pm} = 0.00552$. Since f_{\pm} is a complicated function of x_{\pm} , the simplest method of calculating the latter is by trial and error. The solution is $x_{\pm} = 0.0198$, $r = 0.674$, $f_{\pm} = 0.279$.¹⁵

The determination of the electromotive force of a cell containing a solution of any concentration introduces nothing new since

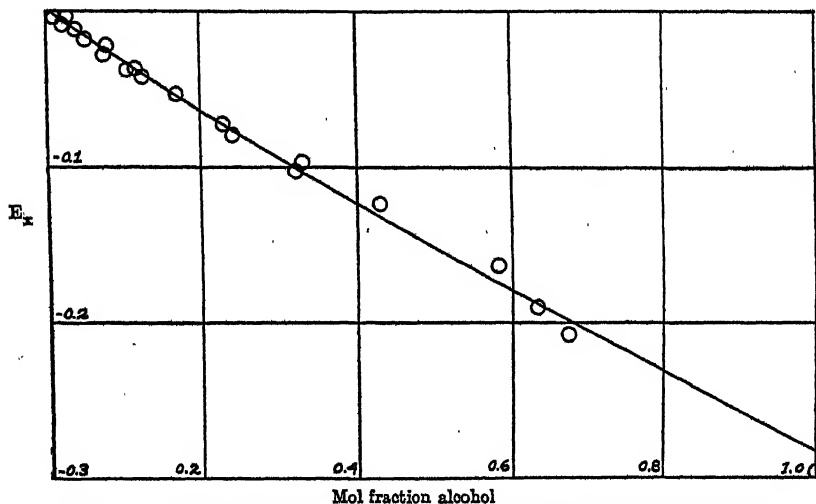


FIG. 2. POTASSIUM CHLORIDE IN WATER-ETHYL ALCOHOL MIXTURES

it requires merely the computation of E_x by the method of the last paragraph, and of $E_x - E$ by the method of the two preceding paragraphs.

Figure 2 shows similar results for potassium chloride. It is less conclusive for there are no very accurate measurements in pure alcohol, so the curve has to be drawn from the intermediate points. The computed radius, b , is 1.35×10^{-8} , only slightly larger than that of sodium chloride.

¹⁵ The volume is computed by adding to the volume of the solvent 0.020 liter for each mol of salt, an approximation which is sufficiently accurate for the computation of f_{\pm} .

In the next case we have at least one effect in addition to the electrical forces. Figure 3 shows E_x for hydrogen chloride in the same solutions, computed by the method given above from direct measurements of electromotive force.⁵ The broken line corresponds to the curves which describe the behavior of the alkali chlorides. It does not agree at all with the experimental points. There is evidence from other sources that in water the hydrogen ion does not exist as H^+ but combines with one molecule of water to form H_3O^+ . The simplest assumption to handle mathe-

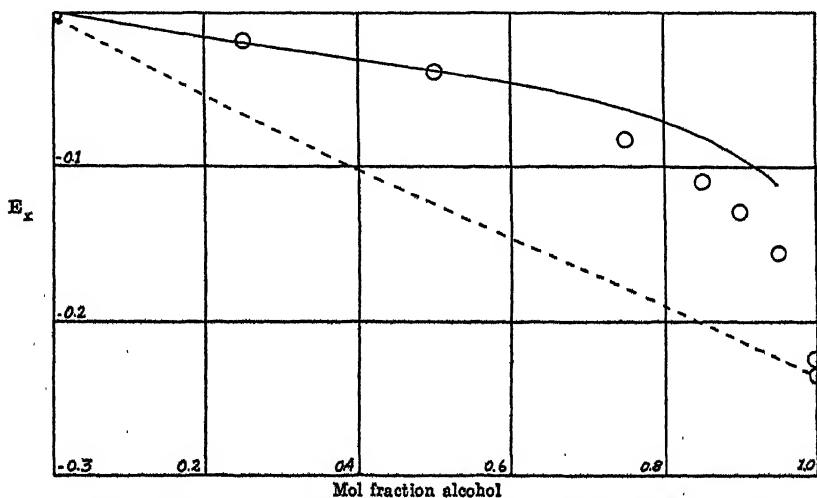


FIG. 3. HYDROCHLORIC ACID IN WATER-ETHYL ALCOHOL MIXTURES

--- Hydrogen ion not hydrated
 — Hydrogen ion hydrated

matically is that this reaction is practically complete even in solutions consisting mostly of alcohol, although it might be nearer the truth to assume that there is some alcoholated hydrogen ion in the latter solutions. Electromotive force measurements give the activity of the simple ion, which may be calculated from that of the hydrated ion and the vapor pressure of water from the solution. The full curve is the theoretical one for a pair of ions of size 5×10^{-8} cm., the size determined from the measurements in water and in 50 mol per cent alcohol. It is carried only to 95 per cent alcohol, for in pure alcohol our assump-

tion becomes absurd. The agreement is only fair, which indicates that our assumption is too simple. In spite of the failure to agree exactly it seems to me that this is the best evidence so far that the hydrogen ion combines with one molecule of water. The evidence is equally good that it does not combine with more than one molecule and that the alkali and chloride ions combine with none at all.

Figure 4 illustrates the effect of another factor. It shows the change in electromotive force, E , of a cell containing 0.1 N HCl

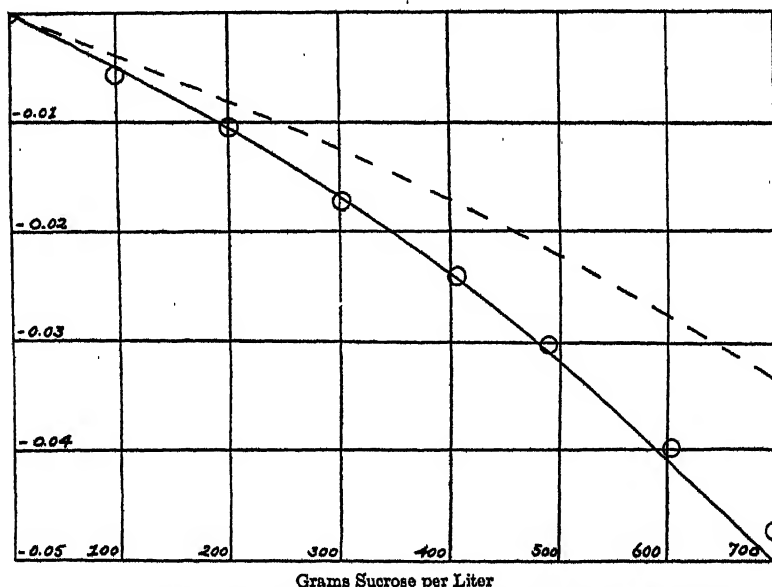


FIG. 4. HYDROCHLORIC ACID (0.1N) IN Water-SUCROSE MIXTURES

--- Sucrose not hydrated
 — Sucrose hydrated

and varying amounts of sucrose.¹⁶ Seven hundred grams of sucrose per liter is only about 6 mol per cent, so that this whole diagram corresponds to a thin slice from the left side of the last one. The circles give the experimental measurements, the broken line corresponds to the one which fits for alcohol solutions. Some years ago I showed that the vapor pressure of water from

¹⁶ Scatchard, *J. Amer. Chem. Soc.*, **48**, 2026 (1926).

sucrose solutions could be accounted for by assuming that all the deviation from the laws of ideal solutions is caused by the formation of a hydrate of sucrose. Taking into account this hydration in computing the mol fraction of the ions gives the full curve which agrees very well with the experiments. The discrepancy of about three millivolts in the most concentrated solution may be due to experimental error for electromotive force measurements, in these solutions are very difficult.

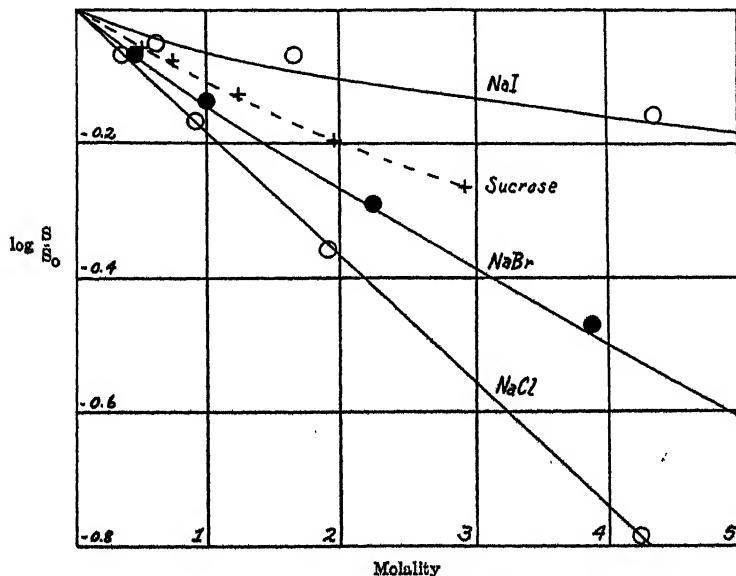


FIG. 5. SOLUBILITY OF ETHYL ACETATE AT 25°

We have seen that the electrostatic forces will account completely for the properties of sodium and potassium chlorides in alcohol-water mixtures. For hydrochloric acid in the same solutions there is the additional effect of the hydration of the hydrogen ion. In sucrose solutions the hydration of the sucrose also shows an influence.

ACTIVITY OF THE NON-ELECTROLYTES

In considering the effect of salts on the activities of non-electrolytes it is not possible to isolate the separate disturbing

factors. Our theory says that the logarithm of the solubility, expressed as mol fraction, of a non-electrolyte is a linear function of the ionic strength in very dilute solutions. Practically this relation holds approximately, and sometimes very accurately, up to very high salt concentrations, but the agreement is probably due to the compensation of several disturbing factors. Figure 5 shows the results for ethyl acetate in water.¹⁷ The curve for sodium chloride is a straight line up to 5 molal. Those for sodium bromide and sodium iodide curve but it is possible to

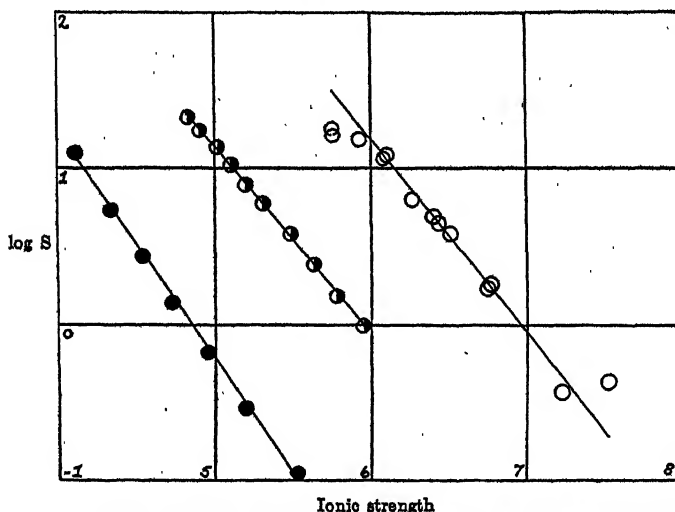


FIG. 6. SOLUBILITY OF PROTEINS IN AMMONIUM SULFATE SOLUTIONS

- Egg albumin (C + M)
- ◐ Egg albumin (S + H)
- Pseudo globulin (S)

determine the value of b from the limiting slope at zero concentration. The dotted line shows the solubility in sucrose solutions and indicates how large the non-electrical effects may become. Probably it represents an extreme case.

Figure 6 shows the effect of ammonium sulfate on the solubility of two proteins.¹⁸ Please note that the change in solubility

¹⁷ Measurements of Glasstone and Pound, *J. Chem. Soc.*, 127, 2660 (1925).

¹⁸ Cohn, *Physiol. Rev.*, 5, 410 (1925). Measurements of Chick and Martin, of Sørensen and Høyrup, and of Sørensen.

is much larger. For the same ionic strength the slope is about ten times as great for pseudoglobulin as for ethyl acetate. A factor of ten in a logarithmic expression is very important. While the solubility of ethyl acetate is reduced one-half, that of pseudoglobulin is reduced to one thousandth.

I have no data on osmotic pressures and the freezing points of electrolyte-non-electrolyte mixtures do not lend themselves to diagrammatic representation in two dimensions. However, the calculated size b remains constant within the limit of error of the most accurate measurements so far available up to salt concentrations of half molal and non-electrolyte concentrations of one molal, the highest concentrations tested. Table 1 gives some

TABLE 1
Freezing points of aqueous sodium chloride-ethyl alcohol mixtures¹⁹

$m_s = \mu$	m_s	e_{n+s}	e_n	e_s	$e_{n+s} - e_n - e_s$	b
0.1274	0.5286	1.422	0.961	0.421	0.040	0.96
0.2430	0.5286	1.841	0.961	0.801	0.079	0.93
0.5038	0.5286	2.776	0.961	1.654	0.151	0.89
0.1851	1.1203	2.662	1.915	0.612	0.135	0.96
0.3082	1.1203	3.146	1.915	1.015	0.216	0.94
0.4969	1.1203	3.891	1.915	1.636	0.340	0.98

results for sodium chloride-ethyl alcohol mixtures in water which show the relative magnitude of the electrostatic effect and also the agreement between different experiments.

THE IONIC RADII

I want next to show you the test of the theory by comparing the sizes of some ions determined by different methods and in different systems. Instead of tabulating the size of the ions directly I have given their reciprocals because the salt effect is directly proportional to this quantity, it shows better the possible experimental error, which is of the order of 0.1 in the figures shown, and because it is this quantity which should be additive for the different ion pairs. Of course the value 1 in this table corresponds to a radius of 1×10^{-8} cm., 0.5 to twice that radius, 0.25 to four times, etc.

The first seven rows of table 2 give a direct test of the theory, and they represent the only systems for which the dielectric constants have been directly measured. The last two are taken as illustrative of a fairly large number of systems for which there are no measurements of the dielectric constant. Hydrogen and oxygen were chosen because it seems most improbable that they should show any specific effect. In these cases the value of

TABLE 2
Reciprocal radii of ions— $10^{-8}/b$

METHOD	HCl	LiCl	NaCl	KCl
E.m.f. and salt solubility ⁵	0.20	0.25 ^{19a}	0.76	0.74
Freezing point, ethyl alcohol ¹⁹			1.06	1.06
Freezing point, ethyl acetate ²⁰		0.75	0.97	0.98
Freezing point, sucrose ²⁰		1.25	0.93	0.75
Solubility of ethyl acetate, 25° ²¹			0.71	0.61
Solubility of ethyl acetate, 25° ¹⁷		0.63	0.70	0.69
Solubility of ethyl acetate, 50° ¹⁷		0.64	0.80	0.85
Solubility of hydrogen, 15° ²²		0.72	(0.76)	0.80
Solubility of oxygen, 15° ²²	0.17		(0.76)	
Solubility of oxygen, 25° ^{23,24}	0.23	0.50	(0.76)	0.72

¹⁹ Sachs, *Thesis*, Mass. Inst. Techn., 1925.

^{19a} Based on the measurements of Pearce and Hart, *Jour. Amer. Chem. Soc.*, **44**, 2411 (1922). Measurements on the same cell by Drucker and Schingnitz, *Z. physikal. Chem.*, **122**, 149 (1926) lead to the value 0.74.

²⁰ Measurements of Rivett, *Medd. K. Vetenskapsakad. Nobelinst.*, **2**, No. 9, (1913).

²¹ Measurements of Lunden, *ibid.*, No. 15 (1913).

²² Measurements of Steiner, *Wied. Ann.*, **52**, 275 (1894).

²³ Measurements of Geffcken, *Z. physik. Chem.*, **49**, 257 (1904).

²⁴ Measurements of McArthur, *J. Physical Chem.*, **20**, 495 (1916).

β may be determined by using the value of b determined for some salt in another system. I have used sodium chloride as the reference salt because it has been included in every series, and because the size is particularly well determined from salt solubility measurements.

The fact that reasonable sizes for the ions are obtained seems to me to give very good confirmation of the theory. The agreement between the values in the different series gives a still stronger confirmation. The values from the freezing points of

ethyl alcohol and of ethyl acetate are relatively large. In these cases the value of β was measured at 18°, and it appears that it increases somewhat as the temperature is lowered to 0°. The other variations may be due to specific effects, but it is entirely possible that they are due merely to experimental error.

Table 3 shows the effect of various potassium salts with constants computed to give the relative salt effect at the same equivalent concentration, decreasing down the column. The three series place the different salts in the same order except for the nitrate and iodide, for which the difference is well within the experimental

TABLE 3
Salt effects of potassium salts at equivalent concentrations
Valence factor $\times 10^{-3}/b$

ANION	VALENCE FACTOR	FREEZING POINT		GAS SOLUBILITY O ₂ or H ₂ 22,23,24
		EtOH ¹⁰	EtAc ²⁰	
HPO ₄ ⁻	1.5	1.32		
SO ₄ ⁻	1.5	1.17	1.00	1.21
Cit. ⁻	2	1.14		
CO ₃ ⁻	1.5			1.10
OH ⁻	1			0.96
HCO ₃ ⁻	1	1.07		
Cl ⁻	1	1.06	0.98	0.76
Br ⁻	1	1.02	0.71	0.65
NO ₃ ⁻	1	0.82	0.58	0.51
I ⁻	1	0.89	0.48	0.43

error. The difference in the magnitude of the change from salt to salt in the different series may be due to small specific effects, but it may also be due entirely to experimental error. It will be noted that table 2 gives a Hofmeister series for the anions with solutes which cannot be colloidal, which are not appreciably affected by the hydrogen-ion concentration, and for which the probability of specific chemical action is a minimum.

THE CONSTANT β

In table 4 I have collected the values of β for various non-electrolytes. β , you will remember, gives the relative depression

of the dielectric constant, the relative effect on salt or water activity, and the relative susceptibility to salt effect. In the third column are values relative to that of hydrogen, which increase to 14 for pseudoglobulin, while urea has a negative value,—the dielectric constants of urea solutions are greater than that of water. The main factor in the change in dielectric constant is the displacement of water by another substance (generally) of lower dielectric constant. The magnitude of the effect depends upon the volume of water displaced and upon the

TABLE 4
Dielectric constants of aqueous solutions

SOLUTE	METHOD	β	REL.	$\beta/\text{M.W.}$	REL.
Hydrogen.....	Solubility	0.05	1	0.025	1
Oxygen.....	Solubility	0.06	1.2	0.0019	0.076
Ethyl alcohol ²⁵	Direct	0.045	0.9	0.0010	0.040
Ethyl acetate ¹⁶	Direct	0.09	1.8	0.0010	0.040
Sucrose ^{26,27}	Direct	0.08	1.6	0.00023	0.0092
Egg albumen.....	Solubility	0.6	12	0.00002	0.0008
Pseudoglobulin.....	Solubility	0.7	14	0.00001	0.0004
Urea.....	Direct	-0.04	-0.8	-0.00075	-0.030

²⁵ Nernst, *Z. physik. Chem.*, 14, 622 (1894).

²⁶ Debye and Sack, private communication.

²⁷ Harrington, *Physical Rev.*, 8, 581 (1916).

dielectric constant of the displacing medium. To test the reasonableness of the values obtained indirectly the comparison should be made for equal volumes. Such a comparison is given approximately, except for hydrogen and possibly oxygen, in the last two columns which give the change per gram. On this basis hydrogen gives the largest effect and the two proteins lie between sucrose and urea, which appears reasonable. Although the data now available are too limited to permit of very certain conclusions, it seems probable that the proteins may be treated by the same simple theory as ordinary solutes, from which they differ only in those properties which may be attributed to the large size of the molecule.

SUMMARY

I think that this much has been established. For the interactions of electrolytes with non-electrolytes, as for the interaction between ions, electrostatic forces must be taken into account. These forces give rise to an effect on the free energies which is proportional to the ion concentration, rather than to its square root, and which depends not only on the valence of the ion but also on a specific property—the equivalent radius. The effect also depends upon a specific property of the non-electrolyte—the molal depression of the dielectric constant. In some cases the simple electrostatic effect accounts completely for the free energy change. In some others the additional factors can be determined and accurately calculated. At present I do not pretend to be able to tell you with precision what the relative effects of different salts are when there are no additional factors, or to be able to predict when the additional factors will be operative. I think that such predictions could be made after the study of a number of systems. The freezing point method seems well adapted to the extension to a large variety of systems and to the development of a higher degree of precision than that attained in the measurements I have used for my calculations.

I trust that I have made it very clear that I am not here as an emissary of the physicists and physical chemists to present you with a new tool which they have perfected, together with complete directions when and how to use it, but that this is a sketch of a field as yet scarcely touched.

SYMBOLS

activity (with subscript)	q = quantity of electricity (see Equation 6)
diameter of ionic collision sphere (without subscript)	R = molal gas constant
constant in equation 20	s = solubility
radius of electrification sphere	T = absolute temperature, freezing point
constant defined by equation 14	Θ = freezing point depression
molal concentration = mols per liter	V = volume of system
ionic strength (molal concentrations)	v = molal volume
dielectric constant	w = molecular weight divided by 1000
d = density	W_e = electrical work for system
Δ = function defined in note 9	w_e = electrical work per ion
E = electromotive force	x = mol fraction
E_e = electromotive force at equal, very small mol fractions	
e = electric charge	
e = charge of hydrogen ion	
F = free energy	
f = activity coefficient, defined by Equation 3	
κ = function defined by Equation 21	
\ln = natural logarithm	
\log = common logarithm	
m = molality = mols per 1000 grams solvent	
μ = ionic strength (molalities)	
N = Avogadro's number	
n = number of mols of a component in system	
ν = number of ions from one molecule of electrolyte	
P = osmotic pressure	
π = ratio of circumference of circle to diameter	
Φ = electrostatic potential at surface of ion	
Q = molal heat of fusion	

SUPERSCRIPTS

'	= first system
"	= second system

SUBSCRIPTS

$_e$	= electrical
$_I$	= corresponding ideal solution
$_i$	= any component
$_{n+s}$	= system containing $n_1 + n_2 + n_3$
$_s$	= corresponding system with ratio n_2/n_1 but with $n_3 = 0$
$_{\cdot}$	= corresponding system with same ratio n_2/n_1 but with $n_3 = 0$
$_o$	= solvent (to indicate properties of pure solvent)
$_1$	= solvent
$_2$	= non-electrolyte solute
$_3$	= electrolyte
$_{+}$	= positive ions
$_{-}$	= negative ions
$_{\pm}$	= mean for positive and negative ions

IMMUNOLOGICALLY SPECIFIC POLYSACCHARIDES

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In the present state of knowledge of the field a review of polysaccharides which react specifically with immune sera may appear somewhat premature. The possibilities and limitations of the subject are barely understood, few of the substances in question have been investigated in detail, and indeed, a rigorous proof has never been given that the polysaccharides isolated are the actual specific substances and not merely adsorbents carrying them. However, the evidence on the last point seems so overwhelming that a new and unsuspected field of sugar chemistry would appear to have been opened up. Moreover, the subject is of such importance to immunology in general and to the understanding of bacterial specificity in particular, that the writer will, perhaps, be pardoned if he attempts to call attention to the data already collected and to such advances as they may have occasioned, leaving final conclusions and final judgment to some future time.

It has long been known that polysaccharides with the properties of gums could be produced through the agency of microorganisms. As early as 1861 Pasteur (1) observed the formation of gum from saccharose solutions when seeded with certain "petits globules reunis en chapelet." The subsequent literature has been reviewed by Buchanan (2), who described a gum which he considered the capsular material of *B. radicola*. The polysaccharide material from the encapsulated, pathogenic Friedländer bacillus was isolated by Toenniessen (3) and later by Kramár (4). None of these investigators ascribed any immunological significance to the gums which they isolated and it is only in the light of the work now to be discussed that their classification with the specific polysaccharides would seem justified.

Following the demonstration (5) by serological methods of the existence of three fixed antigenic type of *Pneumococcus*, Dochez and Avery (6) showed in 1917 that whenever pneumococci of Type I, II, or III are grown in fluid media, there is present in the cultural fluid a substance which precipitates specifically in antipneumococcus serum prepared by injection of animals with a strain of the homologous type. This soluble substance was demonstrated in culture filtrates even during the initial period of rapid multiplication when little or no cell death or disintegration is occurring. Examination of the blood and urine of experimentally infected animals gave proof of the presence of this substance in considerable quantities in the body fluids, showing that this soluble material, elaborated at the focus of the disease, readily diffuses throughout the body, is taken up in the blood, passes the kidney, and appears in the urine unchanged in specificity. Similarly, a study of the serum of patients suffering from lobar pneumonia revealed a substance of like nature in the circulating blood during the course of the disease in man. Furthermore, examination of the urine of patients having pneumonia due to pneumococci of Types I, II, and III, showed the presence of this substance in some stage of the disease in approximately two-thirds of the cases.

In 1923, Zinsser and Parker (7) prepared from filtered alkaline extracts of bacteria of several varieties, including pneumococci, products which they called "residue antigens," which appeared free from coagulable protein and were specifically precipitable by homologous antisera. These observers considered their acid- and heat-resistant antigenic materials analogous to the soluble specific substance of *Pneumococcus* described by Dochez and Avery.

In their earlier studies Dochez and Avery found that the specific substance is not destroyed by boiling; that it is readily soluble in water and precipitable by acetone, alcohol, and ether; that it is precipitated by colloidal iron, and does not dialyze through parchment; and that the serological reactions of the substance are not affected by proteolytic digestion by trypsin. Since the substance is easily soluble, thermostable, and type-

specific in the highest degree, it seemed an ideal basis for the beginning of a study of the relation between bacterial specificity and chemical constitution, and this was accordingly undertaken (8).

The Type II pneumococcus was first selected for study, and the fractionation and purification of the specific substance were followed at each step by means of the precipitin test with sera prepared by immunization of horses against Type II pneumococcus. Eight-day cultures of the organism in meat-infusion phosphate broth were concentrated on the water-bath to about one-fifteenth volume, precipitated with 1.2 volumes of alcohol, and centrifuged. Of the three layers formed, the middle gummy layer contained most of the specific substance, and a large proportion of the accompanying impurities could thus be eliminated in a single step. Further purification depended upon repeated precipitation with alcohol under varying conditions, also by means of saturation with ammonium sulfate and final precipitation by pouring into 10 to 15 volumes of acetone. The yield from 300 liters of culture was 2.5 to 3.5 grams.

It has generally been supposed that substances with immunologically specific properties are derived from proteins. It was therefore an entirely unexpected occurrence that as the precipitin titer of the recovered soluble specific substance rose during the process of purification, the nitrogen content dropped until, after the final acid precipitation, this element remained present only in undeterminable traces. On the other hand, the material isolated took on more and more the properties of a polysaccharide, so that it became evident that a sugar derivative was at least the carrier of whatever might be the true specific substance itself.

Attempts were made to separate this hypothetical specific substance from the polysaccharide, but no significant change in the properties of the material could be effected by precipitation with basic lead acetate, uranyl nitrate, or safranin, or by adsorption on alumina and recovery from this, or even by specific precipitation with a large quantity of immune serum and recovery of the specific substance from the immune precipitate. Furthermore a substance with the properties of a polysaccharide was obtained whether the starting material was the entire broth

culture, the centrifuged pneumococci themselves, or the urine of pneumonia patients. When exposed to the action of 1:1 hydrochloric acid in the cold the specific activity did not diminish appreciably until reducing sugars appeared, so that the specific substance and the polysaccharide, if not identical, appeared at least to be very closely associated. Attempts at a separation by means of polysaccharide-splitting enzymes also failed, as the sugar derivative proved very resistant to this type of hydrolysis.

On acid hydrolysis the soluble specific substance of Type II pneumococcus, in the state of purity outlined above, yielded about 70 per cent of reducing sugars, calculated as glucose. These sugars actually consisted mainly of glucose, as shown by the isolation and identification of glucosazone, and the formation of saccharic acid on oxidation, isolated as the acid potassium salt. Other possible constituents have not yet been identified.

Thus the soluble specific substance of Type II pneumococcus is apparently identified with a weakly acidic, nitrogen-free polysaccharide made up chiefly of glucose units. Its acid equivalent is about 1250 and the specific optical rotation is about $+74^\circ$. It is not precipitated by barium hydroxide or heavy metal salts with the exception of basic lead acetate and uranyl compounds. It reacts at a dilution of 1:5,000,000 with Type II antipneumococcus serum but does not precipitate Type I and Type III antisera at a concentration of 1:400. The substance is converted by acetic anhydride and pyridine into a very sparingly soluble triacetyl derivative.

Little work was necessary on the soluble specific substance of Type III pneumococcus to show that marked chemical differences existed between it and the corresponding substance of Type II. The method of concentration and purification was at first the same, but it soon developed that not only is the Type III specific substance precipitated by heavy metal salts such as those of silver, mercury, and copper, which do not precipitate the Type II substance, but that, in conformity with this, the Type III substance is the soluble alkali or alkaline earth salt of an *insoluble* strong acid which is thrown out of solution in the presence of an

excess of strong hydrochloric acid. This property was most useful in separating the specifically reacting polysaccharide acid from accompanying glycogen or erythrodextrin, derived either from the meat broth or from the bacteria themselves. By repeated purification as the insoluble free acid the Type III specific substance was easily obtained in a state of relative purity without the aid of precipitation by ammonium sulfate and with fewer fractionations from alcohol than proved necessary with the Type II substance. Successive batches also agreed very closely in their chemical and physical properties, indicating that one is apparently dealing here with a much more definite chemical entity than in the case of the Type II substance, in which, in the absence of so characteristic a property which can be made use of in the process of purification, the variation in the findings is over a much wider range.

The Type III soluble specific substance is thus also isolated as a nitrogen-free polysaccharide. It is, however, not a dextro-rotatory, weak acid like the Type II substance, but a levorotatory, strong acid, with an equivalent value of about 340 and $[\alpha]_D$ of about -33° . It is precipitated by barium hydroxide in excess and by heavy metal salts, and is also rendered insoluble by the addition of strong hydrochloric acid. In as high a dilution as 1:6,000,000 it still reacts with Type III antipneumococcus serum. On hydrolysis the substance yields glucose and a disaccharide acid which will be discussed below. No further purification was effected by precipitation with barium hydroxide or by adsorption on highly active alumina prepared according to Willstätter and Kraut (9).

The chief stumbling-block to chemical progress with the bacterial polysaccharides has been the small amount of material available. It has been found possible in the case of the Type III pneumococcus to overcome this objection to some extent. In the first place this microbe produces far more specific substance than do the other two antigenic types of *Pneumococcus*, and the substance is the easiest of the three to isolate and purify. Furthermore, once the properties of the Type III specific substance had

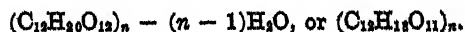
been established it was considered proper to add glucose to the usual *Pneumococcus* broth. In this way a greatly increased growth was obtained, and whereas the yields of specific substance were originally from 6 to 9 grams per 300 liters of broth, they now rose to 35 to 40 grams. With these increased amounts of material it became possible to throw new light on the structure of the polysaccharide and its hydrolysis products (8f).

When the Type III specific substance is partially hydrolyzed in the cold with 75 per cent (by weight) sulfuric acid until it no longer precipitates on dilution, and is then further hydrolyzed with boiling normal sulfuric acid until the maximum reducing power is attained, a small amount of glucose is formed and may be identified through the analysis, melting point, and mutarotation of the osazone, and through oxidation to saccharic acid. By far the largest portion of the hydrolytic products, is, however, precipitated by basic lead acetate and yields a crystalline morphine salt which melts at $153-6^{\circ}$ and shows an initial $[\alpha]_D$ of -47.9° , changing to -54° . This salt, on decomposition, gives rise to an hitherto uncrystallizable sugar acid, differing little from the crude material before purification. Its reducing power is 50 per cent that of glucose; its acid equivalent is found to be 363, while the value calculated for a disaccharide acid, $C_{12}H_{18}O_{10}$. COOH is 356; on prolonged hydrolysis only a small amount of glucose, in addition to unhydrolyzed material, can be isolated, the acid half of the portion hydrolyzed apparently decomposing similarly to glucuronic acid and yielding approximately the expected amount of furfural; the reducing group of this bionic acid is aldehydic, as shown by its quantitative determination by the Willstätter-Schudel method (10); the acid also gives the color reaction with naphthoresorcinol characteristic of the glucuronic acid type, but since the easily isolable mucic acid is not found on hydrolysis and oxidation with nitric acid, the acid portion of the molecule can scarcely be galacturonic acid. Whether the saccharic acid actually isolated from the oxidation mixture arises only from the glucose half of the molecule, or from the acid portion as well, can not be stated at present.

As to the position of the union of the glucose to the sugar acid the evidence at hand does not permit any conclusion. The linkage may be either through the reducing group of the glucose or else the reducing group of the sugar acid is involved in the glucosidic union. This type of union is indicated by the fact that on further hydrolysis the reducing power of the aldobionic acid increases to about 65 per cent before dropping owing to destruction of the hexose-uronic acid liberated.

It is evident from quantitative data obtained on the hydrolysis of the original polysaccharide (8f, p. 412) that the aldobionic acid accounts for about 85 per cent of the total products of hydrolysis, while only 9.5 per cent is glucose, and 5.5 per cent unaccounted for. Now it has also been found (8f, p. 412) that the aldobionic acid itself slowly hydrolyzes, with liberation of glucose to the extent of about 1 per cent per hour on boiling with dilute mineral acid. It is therefore not illogical to assume that the glucose set free during the hydrolysis of the polysaccharide owes its origin, not to a separate part of the carbohydrate molecule, but chiefly to a secondary reaction involving the disaccharide acid. This assumption is all the more justified by the fact that no glucose is split off during the preliminary hydrolysis by 75 per cent sulfuric acid in the cold. Since, also, these partial hydrolysis products show, by their acid equivalents, one carboxyl group for every two sugar nuclei, it would seem that the polysaccharide as a whole is built up of units of the disaccharide acid.

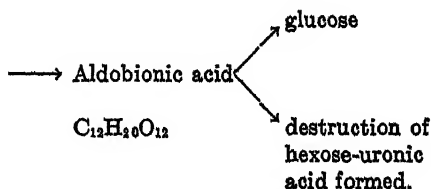
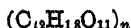
The condensed, or polysaccharide form of a hexose-hexose-uronic acid should have the formula



A substance of this composition should have an acid equivalent of 338 and a carbon and hydrogen content of 42.6 per cent and 5.4 per cent respectively. These figures are practically identical with actual analytical values obtained (cf. Table 1). Thus one may justifiably conceive of the Type III soluble specific substance as a condensation product of the aldobionic acid, $C_{12}H_{20}O_{12}$,

built up in such a way that the reducing groups disappear and carboxyl groups remain free. Hydrolysis by means of acid follows the course:

Polysaccharide acid \longrightarrow Intermediate acids



In view of the evidence collected it is believed that the soluble specific substance of Type III pneumococcus is a definite chemical individual, polysaccharide in nature, and that it is composed of units of a difficultly hydrolyzable aldobionic acid in which glucose and a hexose-uronic acid are combined in such a way that one aldehyde group and the carboxyl remain free. The polysaccharide is thus unusual not only in its possession of the property of immunological specificity, but in its chemical constitution as well.

In the case of the soluble specific substance of Type I pneumococcus (8e) it was again found necessary to modify somewhat the method of isolation, owing to its presence in the culture fluid in relatively smaller amount than are the Type II and Type III specific substances, and because of the insolubility of the product at its isoelectric point, which lies at about pH^4 . It was also found advisable to wash the alcoholic precipitates with 0.5 normal acetic acid, in which the isoelectric Type I specific substance is insoluble, while the accompanying glycogen or erythro-dextrin dissolves. Finally, the substance was precipitated at as low a temperature as possible with strong barium hydroxide solution, reprecipitated by alcohol in the presence of hydrochloric acid, and dialyzed free from chloride ion. The Type I substance is a weak base and precipitates as the excess of hydrochloric acid is removed. The yield from 300 liters was 2 to 3 grams.

The Type I soluble specific substance also appears to be a sugar

derivative, but differs from the other two substances in its lower percentage of sugar liberated on hydrolysis and in containing nitrogen as an apparently essential component. It rotates the plane of polarized light about 300° to the right, is a strong acid and a weak base, and is very sparingly soluble at its isoelectric point, which lies at about pH 4. When in concentrated solution it also separates from hydrochloric acid at an acidity of 1.3 normal, redissolving as more strong acid is added. In spite of a nitrogen content of 5.0 per cent the substance gives none of the usual protein color tests. One-half of the nitrogen is liberated on treatment with nitrous acid and reducing sugars appear at the same time, while the specific reaction vanishes. Under the same conditions the Type II and Type III substances are unaffected by nitrous acid. It would thus appear that this portion of the nitrogen, at least, is an integral part of the specific substance, and is possibly linked to the reducing group of a sugar derivative, as Karrer believes is the case in the polyglucosamines (11). The substance gives the color reaction for glucuronic acid with naphthoresorcinol, but yields mucic acid on oxidation, indicating a relationship to galactose. Since the carbon and hydrogen contents of the substance are close to the theoretical values for polysaccharides it appears possible that in it a nitrogenous sugar derivative is linked to galacturonic acid through the reducing group of the latter. The Type I substance is precipitated by barium hydroxide in excess, by heavy metal salts, and by phosphotungstic acid. In the specific precipitin reaction with Type I antipneumococcus serum it can be detected in dilutions as great as 1:6,000,000, while at a concentration of 1:400 it gives a faint cloud with Type III antiserum.

A preparation derived from Type I pneumococci which had been separated from the accompanying broth by centrifugation and one subjected to additional purification by adsorption on alumina failed to show differences from those isolated as above.

The properties of the soluble specific substances of the three antigenic types of *Pneumococcus*, in the state of purity attained up to the present are summarized in Table 1.

The three polysaccharides contain no sulfur or phosphorus

and differ from the starch-glycogen group of carbohydrates in their acid properties, in giving no color with iodine, and in their resistance to the ordinary carbohydrate-splitting enzymes. Each substance breaks down on hydrolysis into reducing sugars, a part of which, at least, is peculiar to itself. The Type I substance differs sharply from the other two in containing nitrogen and in possessing basic as well as acidic properties, while of the other substances, the Type II is a dextrorotatory weak acid and the Type III a levorotatory strong acid. Especially striking is the

TABLE 1

Soluble specific substances of the three fixed antigenic types of pneumococcus and of Friedländer bacillus (Type B)

TYPE	$[\alpha]_D$	ACID EQUIVALENT	C	H	N	REDUCING SUGARS ON HYDROLYSIS		HIGHEST DILUTION GIVING PRECIPITATE WITH HOMOLOGOUS IMMUNE SERUM
I	+300°		43.3*	5.8	5.0†	calculated as glucose 28	(Galacturonic acid) (Amino sugar derivative)	1:6,000,000
II	+74°	1250	45.8	6.4	0.0	70	Glucose	1:5,000,000
III	-33°	340	42.7	5.3	0.0	75	Aldobionic acid, glucose	1:6,000,000
Frdl. bac.	+100°	685	44.6	6.1	0.0	73	Glucose	1:2,000,000‡

* Theory for $(C_6H_{10}O_5)_n$: C, 44.4 per cent; H, 6.2 per cent.

† Amino N, 2.5 per cent.

‡ Rabbit antiserum.

occurrence of specific substances of such widely differing properties in microorganisms as closely related as are the three fixed types of *Pneumococcus*.

With the possible exception of the Type III substance it is doubtful whether in their present state of purity, each of the specific substances represents a definite chemical compound. However, in the case of the three fixed types of *Pneumococcus* three totally distinct polysaccharides have been isolated from cultures grown in the same medium. Successive preparations

of each specific substance have been quite uniform regardless of the widely different methods employed in the process of purification. Moreover, substances reactive to the same degree with homologous antisera have been derived both from the microorganisms themselves and from autolyzed broth cultures. Furthermore, the only one of these substances hitherto investigated in detail, namely that of Type III pneumococcus, appears to differ in its structure from that of any other known non-nitrogenous polysaccharide.

It is thought that these and other considerations based on the data presented warrant the belief that the three polysaccharides isolated represent the actual specific substances, stripped of at least a large portion of accompanying impurities, and that they do not merely represent inert material carrying an extremely minute amount of the true specific compounds. If this be admitted, it affords a chemical corroboration of the fact of type specificity in *Pneumococcus*, in the discovery of which only biological methods had been used (5).

An extension of the methods of isolation used above to the Friedländer bacillus resulted in the preparation from agar cultures of the so-called "E" strain of this bacillus (now known as Type B (12)) of another polysaccharide with specific properties of the order possessed by the soluble specific substances of the three fixed types of *Pneumococcus* (13). After repeated fractionation by precipitation with alcohol, then with barium hydroxide, and finally with alcohol in the presence of hydrochloric acid, it was obtained as a nitrogen-free strong acid with an equivalent value of about 685, sparingly soluble in water after drying, but yielding soluble alkali salts. The specific optical rotation is $+100^\circ$.

A 1:200 solution is not precipitated by solutions of silver nitrate, copper sulfate, or phosphotungstic acid, but yields precipitates with barium hydroxide and with both neutral and basic lead acetates. It gives no color with iodine. The substance thus differs markedly from the material isolated from the Friedländer bacillus by Toennissen (3) and Kramár (4), as this gave a red color with iodine and yielded galactose on hydrolysis.

The polysaccharide itself is non-reducing, but on hydrolysis with mineral acid yields reducing sugars, among which glucose was shown to be present by isolation of its osazone and by oxidation to saccharic acid.

From Table 1 it will be seen that this substance greatly resembles that of Type II pneumococcus, and the similarity was found to extend even to precipitation of Type II antipneumococcus serum. The Type II substance in its present state of purity, however, is a weaker acid and is not precipitated by barium hydroxide or neutral lead acetate. It is possible that these differences depend only on the presence of different impurities in the two cases, but opposed to this view are the findings that different preparations of each substance, purified by widely different methods, showed great constancy in each case, and that the absorption of agglutinins and precipitins is not reciprocal with the two organisms. That is, Type II antipneumococcus serum, absorbed with Type B Friedländer bacillus, lost its power to precipitate the Friedländer soluble substance, but still precipitated the Type II pneumococcus substance. Similarly, the Type B Friedländer antiserum, absorbed with Type II pneumococcus, precipitated the Friedländer specific substance, but not that of Type II pneumococcus. If the fact that bacteria possess mutual absorptive capacity be accepted as the criterion of their antigenic identity then the failure of the organisms in question to exhibit this property may be taken as further evidence of the lack of identity of the substances involved.

Granted a chemical difference between the two specific substances, it becomes necessary to account for their marked immunological similarity. In the absence of further evidence as to the structural relations of the two substances, which can only be obtained when large amounts of material become available, it seems reasonable to assume that both contain in a portion of the complex molecule the same or a closely similar configuration of atoms. This essential similarity in molecular grouping would then determine the immunological similarity of the two substances.

A striking and probably analogous example of common anti-

genic properties in substances of remote biological origin is furnished by the phenomenon of heterogenetic specificity originally described by Forssman (14), who showed that following the injection of animal tissues of unrelated species common hemolytic antibodies for sheep corpuscles appear. Landsteiner (15) and Taniguchi (16) have shown that such heterogenetic antigens consist of two component parts, one a protein, the other probably a lipoidal substance. Landsteiner and Simms (17) have found that the lipid constituent, although itself practically devoid of antigenic properties, acquires true antigenicity when combined with protein, and that the antibodies thus induced react with the isolated lipid fraction.

The fact that two biologically unrelated organisms, *Pneumococcus* Type II and Friedländer's bacillus (Type B), possess certain similar serological and antigenic properties suggests that examples of heterogenetic specificity likewise occur among bacteria. In the case of the bacteria hitherto studied, however, the specific substance appears to be a polysaccharide, instead of a lipid, and it further appears probable that when the analogous specific polysaccharides of otherwise totally unrelated microorganisms correspond sufficiently in chemical constitution an immunological correspondence also results.

From another strain of Friedländer's bacillus, the type of which has not yet been identified, Mueller, Smith, and Litarczek (18) have isolated carbohydrate-containing material with a nitrogen content of 1.3 per cent, and showed that this product at high dilutions caused precipitation of homologous immune serum.

A polysaccharide with specific properties has also been isolated from the tubercle bacillus and somewhat similar products have been obtained by widely differing methods, on the one hand by Laidlaw and Dudley (19) and on the other by Mueller (20). In the former case the defatted bacilli were treated with weak alkali, and the portion not precipitated by acetic acid or copper sulfate was adsorbed on uranium hydroxide and freed from accompanying glycogen by repeated fractionation with alcohol, while in the latter case the culture concentrate, freed from nucleic acid derivatives, was treated with "antiformin" and fractionated

under varying conditions from alcohol. In both instances a product giving the reactions of a pentose-containing polysaccharide was obtained, dextrorotatory and practically nitrogen-free, and reacting at high dilutions with the sera of animals immunized to the tubercle bacillus. While the material obtained by Mueller showed $[\alpha]_D +24^\circ$ in acid solution and yielded 99 per cent of reducing sugars on hydrolysis, that of the British workers showed $[\alpha]_{Hg\ green} +79^\circ$ and 50 per cent of reducing sugars on hydrolysis. In neither case were the actual sugars identified. The carbohydrate itself appears responsible for the precipitin reaction given by the complex mixture known as tuberculin.

Specifically reacting material, also apparently carbohydrate in nature, has been isolated from yeast by fractionation with alcohol. The product, closely associated with or identical with the yeast gum, precipitates the sera of rabbits immunized against the yeast (21).

The specific polysaccharides discussed up to the present point were elaborated by microorganisms of the most diverse types. The wide distribution of these substances made it seem not improbable that there might occur among higher plant forms other carbohydrates with specific properties. In a series of tests on water-soluble plant gums samples of gum arabic (gum acacia) were actually found which showed some degree of specific activity (22). By fractional hydrolysis with 1:1 hydrochloric acid it was found possible to remove about one-half of the pentose in combination. The recovered gum showed $[\alpha]_D$ about -10° and a specific activity of 100 to 150 times that of the original material. The interpretation of these findings is deferred until further data are available.

It has thus been shown that microorganisms of the most diverse types, and even a vegetable gum yield individually characteristic polysaccharides which behave not merely as the inert carriers of specific substances of undetermined chemical nature, but react as though themselves actually endowed with immunologically specific properties. Whether or not one accept this view it should be of some interest to consider the immuno-

logical significance of the soluble specific substances and their relationship to other cell constituents of microorganisms.

Initial studies of this phase of the subject have been made in the case of *Pneumococcus* (8b, 23, 24, 8d). When pneumococci are dissolved, either with the aid of bile or by repeated freezing and thawing, the resulting solution yields a precipitate of so-called "nucleoprotein" on acidification with acetic acid. While probably a mixture consisting largely of nucleoprotein and mucoid, it still possesses definite immunological properties which differ sharply from those of the soluble specific substance.

In the first place the protein is antigenic, since it gives rise to antibodies on injection into animals, while the soluble specific substance, though reacting specifically with antibodies to the highest degree, is non-antigenic, that is, unable by itself to stimulate the production of antibodies when injected into animals.

Moreover, the protein isolated from any of the three fixed types of *Pneumococcus*, or from a strain of the heterogeneous Group IV, appears serologically the same as that from any of the other types, for not only does it precipitate antisera to the protein of the other types as well as its homologous antiserum, but its homologous antiserum also reacts equally well with the protein isolated from the other types of *Pneumococcus*. Thus this portion of the pneumococcus protein is not type-specific, like the soluble specific substance, but is rather, species-specific.

Before considering further the immunological characters of these two cellular constituents of *Pneumococcus* it may add to the clearness of the discussion to picture the form or pattern of the cell as it relates to the disposition of these substances. Many of the immune reactions participated in by microbes are presumably surface phenomena, and the nature of the reactive material at the periphery of the cell may determine the readiness of response and even the specificity of reaction. *Pneumococcus* is an encapsulated organism, and there are grounds for the belief that the ectoplasmic, or outer, layer of the cell is composed of carbohydrate material which is identical in all its biological characters with the type-specific substance. On the other hand,

the endoplasm, or somatic substance, consists largely of protein which, as previously pointed out, is species- and not type-specific. This protein is possessed in common by all pneumococci while the carbohydrate is chemically distinct and serologically specific for each of the three fixed types. The cell, therefore, may be conceived of as so constituted that there is disposed at its periphery a highly reactive substance upon which type specificity depends. Thus the structure and, as will be pointed out, the morphological integrity of the cell are determinative factors in bacterial specificity.

Immunization with dissolved pneumococci results in the formation of antibodies which precipitate the pneumococcus protein, but not the soluble specific substance. The fact that this specifically reactive carbohydrate is non-antigenic when separated from the other cellular constituents and is capable of inciting antibody formation only in the form in which it is present in the intact cell, forces the conclusion that in the latter instance it exists not merely as free carbohydrate but also in combination with some other substance which confers upon it specific antigenic properties. Immunization with intact bacteria containing this carbohydrate complex elicits antibodies which not only agglutinate the formed cells but precipitate solutions of the carbohydrate isolated from pneumococci of the homologous type. How the specific polysaccharide is combined in the cell, whether with protein or some other constituent is not yet clear, but it is evident that the compound thus formed is the dominant and essential antigen of the cell, and the one responsible for type specificity. It would also appear to be readily dissociated into its components when the cell is dissolved.

The immunological relationships of the protein and carbohydrate fractions of the cell are graphically presented in Table 2, in which S represents the soluble specific substance (carbohydrate) and P the protein of *Pneumococcus*.

It is thus evident that morphological dissolution of pneumococci is accompanied by antigenic dissociation, for sera prepared from filtered solutions of disintegrated cells free of formed elements fail to exhibit any of the dominant type-specific prop-

erties which characterize sera obtained by immunization with whole bacteria. Morphological integrity of the bacterial cell, therefore, is requisite for the expression of its full antigenic power, and the character of the antibody response is determined by the nature of the cell material used for immunization. The injection of suspensions of pneumococci into animals induces the formation of antibodies against S alone or against both S and P separately, depending upon whether or not these suspensions contain only intact cells or a mixture of both intact and dissolved

TABLE 2

PNEUMOCOCCUS AND CELL CONSTITUENTS		ANTIBODIES DEMONSTRABLE IN SERUM						
Material used for immunisation	Effective antigen	Agglutinations	Precipitations		Complement fixation		Specificity	
			S	P	S	P	Type	Species
Intact cells (SP)†.....	(SP)	+	+	—	+	—	+	—
Carbohydrate S‡.....	None	—	—	—	—	—	—	—
Protein P§.....	P	—	—	+	—	+	—	+
Solutions, extracts containing free S and free P.....	P	—	—	+	—	+	—	+
Suspension of intact cells and dissociated cell constituents (SP), free S, free P.....	(SP),P	+	+	+	+	+	+	+

* = Free S, as antigen, does not fix complement with immune horse serum; is active with immune rabbit serum (unpublished data obtained by E. Vollmond).

† (SP) = Carbohydrate and protein combined, antigen of cell.

‡ S = Free carbohydrate, the soluble specific substance of cell.

§ P = Free protein of cell.

cell bodies. Since pneumococci readily undergo autolysis and dissolution, suspensions and indeed cultures of these organisms almost invariably contain not only formed elements, but also more or less of dissociated cell constituents in solution. Therefore, use of suspensions of pneumococci containing both intact cells and the soluble products of cell disintegration yields on immunization not only type-specific antibodies but antibodies reacting with the protein substance which is common to all pneumococci. While the former generally predominate it is the

presence of this protein antibody with its broader zone of activity which is responsible for the confusing cross-immunity reactions occasionally encountered in supposedly type-specific sera, and which has in some instances led workers even to deny the existence of distinct antigenic types of pneumococci. That the two sets of antibodies involved are separate and distinct is shown by absorption tests: the antiprotein reacting bodies in such sera can be removed by absorption with the protein of a heterologous type without diminishing either the titer of specific agglutinins for the homologous culture or the precipitins for the specific polysaccharide of the corresponding type.

A practical result of the above analysis is the clarity with which the conditions are defined for the production of the most sharply differentiated type-diagnostic sera so important in the study and recognition of the etiological agents of pneumonia in human beings. By the application of the knowledge gained from this study it has also been possible for the first time to classify serologically the bacilli of the Friedländer group and to separate them into three main antigenic types and a heterogeneous group, much as in the case of *Pneumococcus* (12). An understanding of the immunological behavior of the non-hemolytic streptococci has also been facilitated by the same type of analysis of the specific factors involved (25).

The elaboration of the type-specific carbohydrate is a specialized function most active in pneumococci rapidly multiplying in the animal body or in suitable culture media. Under these conditions pneumococci exhibit maximal capsular development, exalted virulence, and distinct type specificity. Without further supporting evidence it would of course be hazardous to venture the assertion that all three of these characters are necessarily and causally related to the S-producing function of the cell. However, it is now known (26) that under certain cultural environments pneumococci lose their type specificity and become avirulent, and that these changes are accompanied by a loss of the capacity to elaborate this soluble specific substance.

In 1917 Cole (27) observed that infected exudates and sera containing soluble reactive substances of pneumococci possessed

the property of neutralizing pneumococcus antibodies, and pointed out the significance of this fact in relation to the therapeutic administration of immune serum. Moreover it has recently been reported that the addition of the soluble specific substance of pneumococcus in very small amounts exerts a definite and specific effect in annulling the inhibitory action of the sera of naturally resistant animals on the growth of pneumococci in serum-leucocyte mixtures (28). It has also been reported (29) that injections of the soluble specific substance lower the resistance of mice to infection with partially attenuated pneumococci.

If then, final proof be brought for the conception that the capsular zone of the organism is largely composed of this carbohydrate substance, is part of the defense mechanism of the cell, and is the site of its initial contact with antibody, then these soluble bacterial polysaccharides acquire new significance not only in the serological reactions of the cell, but in the actual processes of infection and immunity in the host.

The specifically reacting polysaccharides also offer much of chemical interest. While it had generally been assumed that only proteins and their derivatives offered the innumerable opportunities of isomerism and subtle changes requisite for substances exhibiting the phenomena of specificity, the discovery of carbohydrates with specific properties is not as astonishing as might appear on first thought. When one considers the number of asymmetric carbon atoms in the pentoses and hexoses, the different possible points of attachment of the lactone bridge, the possibility of α - and β - glucosidic unions at various points in the molecule, and the addition of sugar acids, the analogs of amino acids, to the large number of sugars theoretically capable of entering into the composition of such polysaccharides, it becomes clear that perhaps only among the carbohydrates could another sufficiently large and protean group of substances be found to afford the possibility of specific manifestations.

The development of the field thus opened has barely begun, and it would therefore be idle to speculate on the relationship within this group of specificity to chemical constitution. That

some such relation exists, however, and is reflected by marked chemical changes, has been shown by the isolation of three such different sugar derivatives as the three soluble specific substances of *Pneumococcus* from three such closely related microorganisms as Types I, II, and III pneumococcus. Also significant is the finding that, even in the case of microorganisms of widely differing origin, such as *Pneumococcus* Type II and the Type B Friedländer bacillus, a certain similarity in the chemical nature of their specific polysaccharides is accompanied by a corresponding similarity in the immunological properties of the organisms themselves.

So far but one specific polysaccharide, that of Type III pneumococcus, has been investigated in detail, and it is too soon to form a judgment as to whether or not the polysaccharides with specific properties constitute a chemically separate and distinct group of carbohydrates. In this one instance the structure certainly appears different from that of any known non-nitrogenous sugar derivative, although an analogy to the nitrogen-containing chondroitin sulfuric acid (30) is discernible, and perhaps even a distant relationship to the pectins (31). In this connection the finding of a specifically reacting polysaccharide as a constituent of gum arabic is of interest, and it is not impossible that sugar derivatives with specific properties may occupy a position of biological significance among higher forms of life as well as among bacteria.

Finally it must again be emphasized that it is for the future to determine the validity, the generality, and the utility of the deductions which have already been drawn and the inferences which seem possible. The work done thus far, however, has not only brought to light new substances of chemical interest, but has pointed the way to an understanding of confusing phenomena in the immunological relationships of at least three microorganisms of pathogenic importance. The hope that its possibilities are not yet exhausted would, therefore, seem not unduly bold.

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THE PRINCIPAL CHEMICAL RESEARCHES ON COD LIVER OIL

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HISTORY AND PREPARATION

The literature comprising the chemical studies on cod liver oil is very extensive, and it contains a mass of contradictory assertions. The confusion follows from the fact that samples of this oil may be widely dissimilar, and that the oil of commerce is prepared by divers methods, occasionally from materials other than the liver of *Gadus morrhua*. Moreover, the mystery surrounding its therapeutic action has led investigators into strange fields of speculation. At present the chemical reactions and composition of cod liver oil are just beginning to be understood; it is well, therefore, to distinguish the substantial researches of the past from those which appear inconsequential.

In the ancient method of preparing fish liver oils the livers are submitted to natural disintegration, with or without the aid of solar or artificial heat, and the oil which separates above the tissue mass is decanted. The early stages of this "rotting process" are, I believe, autolytic rather than putrefactive, for the oils possess considerable bactericidal power, and the enzymes of fish liver obviously must be active at low temperatures.

Dubail (1854) described the methods of rendering once in vogue at the principal fishing grounds of the world. The rotting process was in general use, but he stated that the London apothecaries in 1849 employed a steam-jacketed apparatus, and that shortly thereafter one Dr. Fleury of Newfoundland devised a tinned copper cooker for rendering the oil without decomposition. He added that Fleury had studied the storage of oil in the cod-fish liver—the remarkable fattening which follows the early

summer feeding migration. Dubail failed to mention that Fox (1848), possibly as agent of the London pharmacists, established at St. John's the first factory for the manufacture of "steam process" oil. Möller (1853) introduced this process into Norway. He used "a water bath of large dimensions." Berthé (1855) observed, apparently to his surprise, that the steamed oil gave the sulfuric acid color reaction of Goble (1844) just as did rotted oil.

In the modern manufacture of medicinal oil direct steam has all but universally replaced the steam jacket. Fresh livers are steamed for 20 to 30 minutes, and, as the heating is rapid and uniform, enzymic decomposition is practically nil. The oil which separates is filtered from suspended coagulated protein, and chilled to remove "stearine." Nearly all phases of the cod liver oil industry not covered in Möller's book (1895) have been described in the important papers of Drummond and Zilva (1922), Zilva and Drummond (1923), André (1923), and Drummond (1924). The therapeutic history was reviewed by Bennett (1841), Dubail (1854), and Guy (1923).

The early studies on rotted oil were amply considered by Möller (1895), who discussed more than fifty compounds alleged to occur in this material. I shall make little attempt to interpret these investigations, for the reason that the rotted product is really not cod liver oil, but a more or less generally adulterated decomposition product thereof. It is now seldom used in medicine, being confined to technical arts under the name of "cod oil."

Cod liver oil of the best grade ranges in color from golden to pale yellow. It is *devoid of taste-qualities*, but it exhibits a faint, not unpleasant, fishy smell, especially noticeable when the oil is held in the mouth. (The sense of smell is popularly confused with taste.) Even the best oil is subject to considerable variation in composition, the result of seasonal, sexual, nutritional, or other differences in the fish or mode of rendering. The numerous determinations of the physico-chemical constants of cod liver oil, published in every treatise on fats, serve but to

emphasize its indefinite nature. The behavior of cod liver oil with 250 organic solvents has been studied by Bills (1926).

THE FATTY ACIDS

According to Tolman (1909) most marine oils are chemically similar,¹ and very different from vegetable oils and the oils of land animals. Cod liver oil consists of about 99 per cent of simple and mixed fatty acid glycerides and 1 per cent of unsaponifiable matter. It is usually "frozen" ("refined") at a few degrees of frost to remove the high-melting fats which are technically, but erroneously termed "stearine." The "stearine," according to Heyerdahl (1895) and Lewkowitsch (1922) contains much unsaturated material, although it is less unsaturated than the original oil. The existence in the oil of certain fatty acids is well established; that of others is doubtful; no chemist in recent times has attempted a quantitative separation.

Palmitic acid, $C_{16}H_{32}O_2$. Passing over the questionable "gadic acid" of Luck (1856), one finds that Heyerdahl (1895) demonstrated palmitic acid among the fatty acids liberated upon the acidification of soap prepared from refined cod liver oil. The palmitic acid separated at ordinary temperature from the total fluid acids, and was purified by recrystallization from alcohol. The analytical data and the melting point, 62° , clearly identified the product.

Jecoleic acid, $C_{19}H_{38}O_2$; *Fahrion's acid*, $C_{17}H_{34}O_2$. Jecoleic acid, the existence of which has been questioned by Bull (see below), was investigated by Heyerdahl in the following manner. The fluid acids remaining after the separation of crude palmitic acid were oxidized by concentrated alkaline permanganate at 0° . The insoluble oxidation product was washed with cold water, and recrystallized from chloroform to a melting point of 114° to 116° . Ultimate analysis and the acid value indicated the formula $C_{19}H_{38}O_4$ for the oxidized acid, and acetylation indicated two hydroxyl groups, thus: $C_{19}H_{38}O_2(OH)_2$. This

¹ A few exceptions were noted, mainly in the unsaponifiable matter.

compound, "dihydroxyjecoleic acid," may be presumed to have arisen by the oxidation of the jecoleic acid, $C_{19}H_{38}O_2$, originally present in the oil. Dihydroxyjecoleic acid may be compared with the "asellic acid," $C_{17}H_{34}O_4$, m.p. 116° , previously obtained by Fahrion (1893) by somewhat similar methods. Asellic acid presupposes the existence in cod liver oil of an unnamed acid, $C_{17}H_{32}O_2$.

Therapic acid, $C_{17}H_{28}O_2$. The fluid fatty acids were brominated in cold acetic acid. The purified, alcohol-insoluble bromine compound was found to be an addition product, $C_{17}H_{26}O_2Br_2$, of an acid, $C_{17}H_{28}O_2$, originally present in the oil. Heyerdahl called this "therapic acid" because of its supposed therapeutic virtues. Heiduschka and Rheinberger (1911) prepared from the mixed fatty acids a tetrachlorotetraiodide (instead of the octobromide), and dehalogenated this with zinc and acetic acid. Apparently the reduction did not stop at therapic acid, but continued to a dihydrotherapic acid, $C_{17}H_{26}O_2$. Lewkowitsch (1921), who is always skeptical of odd-carbon fatty acids, pointed out that Heiduschka and Rheinberger's analytical data agree better with C_{18} than C_{17} . He believed that therapic acid is nothing but impure clupanodonic acid (see below).

Myristic acid, $C_{14}H_{28}O_2$. For the identification of several acids we must refer to the important work of Bull (1906). With a special apparatus he distilled the methyl esters prepared by the sodium methylate method from pure Lofoten oil. Eighty per cent of the esters could be distilled up to 240° at 10 mm. Hg, and it was found that these esters fractionated themselves fairly definitely at several narrow temperature ranges. The fraction, 161.5° to 165° , upon recrystallization from alcohol, yielded the pure methyl ester of myristic acid. M.p. = 19° . Saponification number = 231.8 (theory = 231.4). The next fraction, 185° to 186° , contained much methyl palmitate, which was also crystallized from alcohol. M.p. = 29.5° . Saponification number = 207.4, exactly theoretical. By saponification palmitic acid was obtained, m.p. = 62° , thus confirming Heyerdahl.

Palmitoleic acid, $C_{16}H_{30}O_2$. The alcoholic solution from the 185° to 186° fraction was saponified, converted into the barium

salt, the latter crystallized from ether, and purified as the zinc salt. The free acid melted at -1° and corresponded to the formula $C_{15}H_{30}O_2$. Bull did not name this acid; the designation, "palmitoleic," was conferred by Lewkowitsch (1906). André (1923) asserts that Bull has recently proposed to call this substance "zoomarinic acid," because of its somewhat general occurrence in marine animal oils.

Stearic acid, $C_{18}H_{36}O_2$. The fraction, 205° to 206° was saponified, and the potassium salt converted into the lead salt. On long standing an ether solution of the lead salt precipitated a small quantity of lead stearate.

Oleic acid, $C_{18}H_{34}O_2$. The ether-soluble portion of the lead salt yielded an acid solidifying at 4° . The acid number, the iodine number, and the solubility ratio of the sodium and potassium salts in alcohol indicated that this was oleic acid.

Gadoleic acid, $C_{20}H_{38}O_2$. The fraction, 223° to 225° was saponified, and the free acid prepared. This was exactly half neutralized with KOH, and twice recrystallized from alcohol. The liberated free acid melted constantly at 24.5° . The analysis, acid number, and iodine number agreed with the formula $C_{20}H_{38}O_2$. This acid, named "gadoleic," occurs in large amount. Bull's excellent distillation curve gave no evidence of Heyerdahl's jecoleic acid. Gadoleic acid oxidized with alkaline permanganate at 0° yields an acid which Bull termed "dihydroxygadic acid" (not related to Luck's gadic acid, however). Lewkowitsch, 1921, calls this more appropriately "dihydroxygadoleic acid." One may suspect that Heyerdahl's dihydroxyjecoleic acid, m.p. 114° to 116° , is a eutectic mixture of dihydroxygadic acid, m.p. 127.5° to 128° , and dihydroxystearic acid, 141° to 143° (the oxidation product of oleic acid), in which case "jecoleic acid" becomes a mixture of gadoleic and oleic acids. It is indeed true that odd-carbon higher fatty acids are extremely rare,² although inseparable eutectic mixtures of fatty acids are not uncommon.

² According to Lewkowitsch (1921) the identity of possibly only daturic acid, $C_{17}H_{34}O_2$, has been established beyond reasonable doubt.

Erucic acid, $C_{22}H_{42}O_2$. The liberated acid of the final fraction, 239° to 240°, was recrystallized from alcohol. It melted sharply at 34°, and gave an acid number of 165.4 (theory = 165.9). The solubility of the salts further indicated erucic acid.

Eicosapentenoic acid, $C_{20}H_{38}O_2$; *Eicosahexenoic acid*, $C_{20}H_{36}O_2$. More recently Bull (1917) prepared a mixture of the bromides of unnamed fatty acids which, he believes, represents $C_{18}H_{34}O_2$ and $C_{20}H_{38}O_2$. The former may be identical with the acid later designated "eicosapentenoic" by Brown and Beal. The latter I have not found elsewhere described, but in keeping with modern nomenclature it might be termed "eicosahexenoic acid."

Hexadecatrienoic acid, $C_{16}H_{28}O_2$; *Clupanodonic (octodecatrenoic) acid*, $C_{18}H_{32}O_2$; *Arachidonic acid*, $C_{20}H_{38}O_2$; *Docosapentenoic acid*, $C_{22}H_{40}O_2$; *Docosahexenoic acid*, $C_{22}H_{38}O_2$. Brown and Beal (1923), in their study of the unsaturated fatty acids of menhaden oil, had occasion to examine also salmon, herring, and sardine body oils, and cod liver oil.³ The saponification numbers, iodine numbers, and refractive indices of all five oils were very similar. Each oil was esterified by the methyl alcohol-hydrogen chloride method, and the esters were fractionally distilled at 15 mm. Hg over 10° ranges. From the iodine number, polybromide number, and percentage of bromine in the bromides of the fractions it was stated that these oils were "decidedly similar in character." The menhaden ester fractions were worked up more thoroughly than the cod liver esters, but it was assumed, on the basis of the indicated similarity, that the cod liver oil possessed essentially the same composition as the menhaden.

Further analytical procedures applied to the *menhaden* oil indicated the presence of several series of acids: C_{14} , C_{16} , C_{18} , C_{20} , and C_{22} , thus confirming Twitchell (1917). Roughly, the degree of unsaturation increases with the molecular weight. Bull's finding of myristic acid, and Heyerdahl's and Bull's recovery of palmitic acid were supported. Methyl clupano-

³ A dark amber, American cod oil—personal communication.

donate was obtained in a state of purity believed to be hitherto unequalled. Unfortunately, the literature on clupanodonic acid is confounding. Tsujimoto (1906) gave this name to an acid, $C_{18}H_{32}O_2$, found in sardine oil. Later (1920, 1922) he decided that the formula should be $C_{22}H_{34}O_2$. In the meantime, however, other investigators perpetuated the designation, clupano-

TABLE 1

ACID	FORMULA	MOST PROBABLE MELTING POINT	ATOMS OF HALOGEN ABSORBED
Myristic.....	$C_{14}H_{28}O_2$	53.8°	0
Palmitic.....	$C_{16}H_{32}O_2$	62.6°	0
Palmitoleic.....	$C_{16}H_{30}O_2$	-1°	2
Stearic.....	$C_{18}H_{36}O_2$	69.3°	0
Oleic.....	$C_{18}H_{34}O_2$	12°-17°*	2
Clupanodonic (octodecatetrenic).....	$C_{18}H_{32}O_2$		8
Gadoleic.....	$C_{20}H_{38}O_2$	25.4°	2
Erucic.....	$C_{22}H_{42}O_2$	33°-34°	2
Docosapentenoic.....	$C_{22}H_{34}O_2$		10

* Dimorphous.

TABLE 2

ACID	FORMULA	ATOMS OF HALOGEN ABSORBED
Hexadecatrienoic.....	$C_{18}H_{30}O_2$	6
Fabrion's.....	$C_{17}H_{32}O_2$	2
Therapic.....	$C_{17}H_{30}O_2$	8
Jacoleic.....	$C_{19}H_{36}O_2$	2
Arachidonic.....	$C_{20}H_{38}O_2$	8
Eicosapentenoic.....	$C_{20}H_{30}O_2$	10
Eicosahexenoic.....	$C_{20}H_{28}O_2$	12
Docosahexenoic.....	$C_{22}H_{32}O_2$	12

donic, for the original $C_{18}H_{32}O_2$, which nomenclature is quite properly retained by Brown and Beal. Hexadecatrienoic, arachidonic, eicosapentenoic, and docosahexenoic acids were probably present. The presence of arachidonic acid in cod liver oil may be indicated in a very early report by Bull (1899). Docosapentenoic acid, $C_{22}H_{34}O_2$, was also found, and its existence, in cod liver oil, was almost contemporaneously confirmed

by Tsujimoto and Kimura (1923). The Japanese authors, however, called this acid *clupanodonic* (*sic*); they prepared it by methods similar to those of Brown and Beal.

In passing it may be noted that all the known fatty acids of cod liver oil fall into the broad series, $C_nH_{2n-2x}O_2$, where $n = 14$ to 22, and x , the number of double bonds, = 0 to 6. That there are many unfilled places in this series is not remarkable, for the iodine number of cod liver oil may itself vary well over 70 units, and the unsaturated acids are rather unstable. Those acids the existence of which is reasonably well established are listed in table 1.

The presence of the acids listed in table 2 is less well established.

"MORRHUIC ACID" AND CHEMOTHERAPY

Recent chemotherapeutic investigations on unsaturated fatty acids have revived the term, "morrhuc acid," in a new meaning. Originally this name was given by Gautier and Mourgues (1888) to a nitrogenous putrefaction product, hydroxydihydropyridine butyric acid, $C_5H_{13}NO_3$, found in rotted oil. Lately, in its new sense, "morrhuc acid" or "sodium morrhuate" is merely an elegant synonym for cod liver oil soap prepared for intravenous injection by the method of Rogers, Muir, Knowles, Cochrane, Davies, and Brierley (1919), Ghosh (1920), or Cutting (1926). While a therapeutic dose of the soaps thus prepared would, I believe, contain an insignificant amount of vitamin D, possibly also vitamin A, the basis for the alleged value of the soap in the treatment of tuberculosis and leprosy must be sought elsewhere.

It is well known that the fat of animals is modified by the fat of their diet. Bell (1851) wrote that pigs which ate cod livers became very fat, and their fat acquired the color and odor of cod liver oil. Channon, Drummond, and Golding (1924) observed that the butter fat of cows receiving cod liver oil tended to acquire the constants of the oil. Williams (1912a, 1912b), perhaps influenced by Heyerdahl's theories, attributed the beneficial effect of cod liver oil to the direct action of the unsaturated acids absorbed in the tuberculous tissues. Lansberg (1919) observed that tubercle bacilli disintegrate in cod liver oil, but



not in vegetable oils. Lindenburg and Pestana (1920, 1921) observed that cod liver oil, like chaulmoogra oil, inhibits the growth of cultures of the leprosy and tubercle bacilli. Ghosh (1920) found that cod liver oil "morrhuate" exhibited the same action in leprosy as the sodium hydnocarpate prepared from chaulmoogra oil, and that it furthermore appeared to exert some action in tuberculosis. Ghosh attributed the action of both cod liver oil and chaulmoogra oil to the highly unsaturated acids. (However, no cyclic acid of the chaulmoogric-hydnocarpic series has yet been recognized in cod liver oil.) The following additional medical references are of interest in this connection: Rogers (1919), Biesenthal (1920), Tambe (1920), Campbell and Kieffer (1922), Tewksbury (1922), Fine (1922), Hume (1924), Grigaut and Tardieu (1924), Pernet, Minvielle, and Pomaret (1925), Caussade, Tardieu, and Grigaut (1925), Jessel (1925). It must be stated, however, that the chemotherapeutic action of cod liver oil has not been thoroughly substantiated. Perhaps, after all, the value of the oil lies mainly in its remarkable content of vitamins.

OXIDATION AND HYDROGENATION

It is evident, from the number of unsaturated acids in cod liver oil, that many hydroxy acids can exist. However, Heyerdahl (1895) has shown that perfectly fresh oil is "hydroxyl-free." On exposure to air it becomes rancid, the rancidity being due to the gradual formation of hydroxy-fats, not to the liberation of fatty acid. The free fatty acids, indeed, oxidize with great ease, but medicinal oil contains only a fractional percentage of acid not combined as glycerides.

Lund (1925) confirmed Heyerdahl's observation that rancidity is caused by oxidation, and proceeded to investigate the mechanism of the oxidative process. By means of the guaiacum reaction he detected organic peroxides during the initial stages of oxidation. These peroxides *per se* imparted no rancidity to the oil; moreover, a rancid oil long after contact with oxygen showed no peroxide. But a non-rancid oil, containing its own peroxide or an added varnish, became rancid on standing, even

in the absence of oxygen. On the other hand, in the presence of an easily oxidized substance, such as liver *débris* or pyrogallol, the rancidity was delayed. It is evident, therefore, that the oxidation of cod liver oil is an autocatalytic process, accelerated by organic peroxides, and retarded by foreign substrates.

In keeping with that of Lund is the earlier work of Drummond, Zilva, and Coward (1924) on the spontaneous deterioration of cod liver oil emulsions. They found that oil emulsified with Irish moss or gum acacia, which contain oxidases, gradually lost its vitamin A potency. On the other hand, gum tragacanth containing no oxidase caused no appreciable destruction. Other evidence of oxidation induced by emulsifying agents appears in the work of Krauss (1914).

The general subject of the autoxidation of unsaturated fatty acids has been reviewed by Fahrion (1920, 1921). He showed that various degrees of oxidation may take place, that oxidation may be accompanied by polymerization or dehydration, and that in certain cases only volatile products may ensue. Kugel-mass and McQuarrie (1925) observed that cod liver oil during oxidation gave off an emanation, probably a vaporous substance, which fogged photographic plates. Servais (1903) detected a malodorous, volatile aldehyde among the products of oxidation by air.

Oxidation becomes a practical problem in the manufacture of cod liver oil. The formation of oxidation products, like those of enzymic hydrolysis, may be satisfactorily avoided only by conducting the rendering operations expeditiously. Other procedures, such as the "gassing" processes that have been exploited during the past 70 years, are, as Lewkowitsch (1922) pointed out, superfluous. Gassing originated in the British patent of Murray (1853) which specified that the "unpleasant odours or fetid flavours" of (rotted) oil are improved by treating the oil with carbon dioxide. Gas exerts no protective influence on cod liver oil which has been properly rendered and bottled.

By hydrogenating cod liver oil edible hard fats are prepared in Norway. The commercial hydrogenation processes unfortunately destroy the vitamins. Schuck (1918), Marcelet (1921),

and Grosser (1925) observed that the fishy smell of cod liver oil disappears during hydrogenation. Schuck found that the highly unsaturated fats, to which the smell is largely due, are reduced by hydrogen even in the absence of a catalyst. His patented process nevertheless destroys the vitamins.

INORGANIC CONSTITUENTS

Years ago considerable importance was attached to the inorganic composition of cod liver oil. For example, in 1853 the *Académie Impériale de Médecine* entertained a special report on whether the oil contains a trace of phosphorus. The nineteenth century literature yields many other discussions of a similar character, some of which were reviewed by Möller (1895). The original references are mainly of historical interest. Since Möller's time the literature has increased disproportionately to the importance of the subject. After examining some forty references I believe that the available information may be accurately digested in a few sentences.

Direct ignition of small quantities of pure cod liver oil yields no weighable ash. Some of the inorganic substances may be determined by carbonizing the oil or its soap, and extracting the char; others may be identified in the unsaponifiable fraction, or in Kjeldahl digests. Iodine is undoubtedly present to the extent of 0.0001 to 0.0005 per cent. Traces of Cl, Br, P, N, Zn, organic S, and alkalies may sometimes be found. Rotted oil may contain any of these elements, and especially N and P, in amounts considerably larger than traces. Certain pharmacologically active nitrogenous bases are characteristic of rotted oil.

UNSAPONIFIABLE FRACTION—VITAMINS

The unsaponifiable fraction of cod liver oil consists of approximately half cholesterol and half pigment, vitamins, hydrocarbons, complex alcohols, and unidentified matter.

Cholesterol. Allen and Thomson (1881) saponified the oil with alcoholic sodium hydroxide, evaporated the soap to dryness with sodium bicarbonate and sand, and extracted the dry mass with petroleum ether. Cholesterol was isolated from the extract.

Cholesterol is a secondary olefinic terpene alcohol, $C_{27}H_{46}O$, melting in the neighborhood of 148° , and giving a great number of derivatives in keeping with its structure.

Pigment. The early assumption, based on the sulfuric acid color reaction, that the pigments of cod liver oil are biliary substances, was doubted by Buchheim (1875). He demonstrated that aqueous extracts of the oil contain no gall-substance, that an emulsion of the oil and bile completely separates on standing, and that the principal bile pigment indeed does not give exactly this color reaction. Salkowski (1887) studied the pigment by means of the improved color reaction of Hager (1885). This test, now called the "Hager-Salkowski reaction," consists in adding to a chloroform solution of the substance to be examined a few drops of concentrated sulfuric acid, whereupon the color evoked passes into the chloroform stratum. Cod liver oil itself gives a light violet color, gradually changing to reddish brown; cholesterol, a blood red, slowly darkening; the purified fatty acids, a dark brownish red; and the concentrated pigment from the oil, an intense indigo blue, rapidly changing to purple violet. Thus the color reactions of cod liver oil may correspond, not to one substance, but to several conjointly—a fact of significance to investigators in search of specific vitamin reactions. Salkowski concluded that the pigment of cod liver oil belongs to the group of "lipochromes." Rosenheim and Drummond (1920) claimed that the substance which gives this lipochrome reaction may be distinct from carotene (carotin) and xanthophyll and any other known lipochrome. Palmer (1922) regarded this lipochrome as not a carotinoid. In private correspondence he writes that the pigment does not give the ferric chloride test for carotinoids. Moreover, it does not disappear when cod liver oil is heated with oil-bleaching charcoal. In fact, the oil thus becomes green, just as *bilirubin* becomes green upon oxidation to biliverdin. De Kadt (1920) reported that when the lipochrome is removed from cod liver oil by the siliceous adsorbent, "tonsil," the treated oil no longer gives the sulfuric acid test, while a carbon disulfide extract of the adsorbent exhibits the lipochrome in concentrated

form. Whether the tonsil removes cholesterol and the vitamins is not known.

Vitamin A and vitamin D. It is not within the scope of this review to discuss the very extensive literature of the fat-soluble vitamins, this field having recently been covered by McCollum and Simmonds (1925), Drummond, Channon, and Coward (1925), and others. Briefly, it may be stated with regard to cod liver oil that biological assays indicate in all samples the presence of vitamins A and D to a greater or less extent. The xerophthalmia-preventing factor (or factors?) known as vitamin A was recognized in this oil by Osborne and Mendel (1914); and the calcification-promoting, rickets-preventing vitamin D by McCollum, Simmonds, Becker, and Shipley (1922). Both vitamins influence growth.

Neither vitamin has yet been isolated, but potent concentrates of one or the other have been prepared by several investigators, notably Zucker (1923), Dubin (1925), Takahashi, Nakamiya, Kawakami, and Kitasato (1925), and Drummond, Channon, and Coward (1925). The general procedure for concentrating the vitamins may be summarized: Cod liver oil, or an extract thereof obtained by means of an immiscible solvent such as alcohol or acetic acid, is saponified with alcoholic alkali. The unsaponifiable matter is extracted by a suitable organic solvent, either from the semi-aqueous solution of the alkaline soap, or from an insoluble precipitate of calcium or magnesium soaps. The crude extract is then further concentrated by fractional crystallization, high-vacuum distillation, treatment with digitonin, and other special operations.

Various researches would indicate that vitamin A originates in green plants, reaching the liver of the cod through the intricate stages of marine nutrition. It is often associated, but certainly not identical, with the lipochromes. It is resistant to saponification, *gentle* hydrogenation, and acetylation, is easily oxidized, is destroyed by short light waves, is volatile with steam or high vacuum, is dialyzable through rubber, is not precipitated by digitonin, may be of an aldehydic nature, and may be responsible for certain color reactions of cod liver oil.

The origin of vitamin D is unknown. It may be exogenous, like A, or endogenous, as by enzymic synthesis within the fish. Vitamin D is resistant to saponification and *gentle* hydrogenation, is only less readily oxidized than A, is destroyed by short light waves, may be dialyzable through rubber, is not precipitated by digitonin, is destroyed by nitrites, and may be related to the sterols. Substances exhibiting antirickettic properties are produced by the action of ultraviolet rays on cholesterol, sitosterol, and ergosterol; and by the action of Florida earth on cholesterol.

Hydrocarbon. The presence in pure cod liver oil of a small amount of hydrocarbon was demonstrated by Drummond, Channon, and Coward (1925), who obtained an unsaturated material, probably squalene, $C_{30}H_{50}$, by the high-vacuum distillation of the unsaponifiable fraction. Recent investigations have shown that squalene is undoubtedly identical with spinacene.

Alcohols. Drummond, Channon, and Coward further report that the unsaponifiable fraction appears to contain a considerable amount of one or more unsaturated fluid alcohols, and a small amount of a saturated solid alcohol melting at 60° (impure), which is possibly batyl alcohol, $C_{20}H_{42}O_2$. Weidemann (1926) believes that the unsaturated alcohols are mainly dihydric, and more unsaturated than selachyl alcohol, $C_{30}H_{40}O_2$, which they otherwise resemble.

IDENTIFICATION AND ASSAY

A great many tests, based on physical and chemical properties, have been proposed for the identification of medicinal cod liver oil and the detection of adulteration, but almost every one of these is, by itself, liable to misinterpretation. The following tests are, in my opinion, the most practical.

1. The oil should be golden to pale yellow in color, and possess an inoffensive, fishy smell.

Fish body-oils, and rotted cod oil, unless bleached and deodorized, usually have a dark color and strong smell. Vegetable oils lack the fishy odor. Blubber oils, foreign liver oils, and bleached oils may escape this test.

2. If to a few cubic centimeters of genuine cod liver oil in a test tube, a similar quantity of the test-sample at the same temperature be added, and partially mixed, no well-defined diffusion striæ should appear.

In this test, originated by Merz (1876), for recognizing the purity of any oil, diffusion striæ must be distinguished from color striæ. Rotted cod oil cannot always be distinguished from steamed, but extensive adulteration by almost any foreign oil is fairly well revealed. Diverse samples of pure cod liver oil produce only feeble striæ on mixing.

3. The Hager-Salkowski color reaction, performed in accordance with the U. S. P. X should be positive, as described.

Consideration of the work of Liverseege (1904), Thaysen (1914), Bohrisch (1918), De Kadt (1920), Poulsson and Weidemann (1923), and Holmes (1924) leads to the conclusion that this long-known reaction may be given by several fish liver oils, and that occasionally cod liver oil fails to give it. However, there is good evidence that such abnormal cod liver oil must have been exposed to conditions of oxidation, insolation, bleaching, etc., which are likely to be detrimental to the vitamins. This test, therefore, is of value principally as a means of determining that the oil has not been improperly treated.

These tests afford no information on the vitamin potency of a cod liver oil. To gain such information, methods of biological assay have been devised which consist in determining the minimum of oil sufficient to cure or prevent certain avitaminoses. The U. S. P. X describes a procedure, based on the growth-resumption method of Zilva and Miura (1921), for the assay of vitamin A. The official standard is low. For the cure of xerophthalmia a larger dose of oil appears to be required than for the resumption of growth. For the assay of cod liver oil for vitamin D the most satisfactory procedure appears to consist in determining the percentage of oil which must be incorporated in a rickets-producing diet to render it curative of rickets. When a large number of assays are performed the vitamin D potency of medicinal oils may be found to vary six or eight times, while the variation in vitamin A may be considerably greater.

Several attempts have been made to estimate these vitamins, particularly vitamin A, by color reactions. The work of Drummond and Watson (1922), Poulsson and Weidemann (1923), Sjörslev (1924), Rosenheim and Drummond (1925), Fearon (1925), Carr and Price (1926), Willimott and Moore (1926), and Rosenheim and Webster (1926) indicates that cod liver oil contains a chromogenic substance which is associated, but not necessarily identical, with vitamin A. Cocking and Price (1926) compare the several reactions, and conclude that the color developed by antimony trichloride affords the most promising results. However, it is true that the value of all the color reactions proposed for the estimation of vitamins is at present highly problematical. Color reactions for vitamin D have not as yet been developed even to the point of extensive parallelism between the tests themselves and the biological findings.

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